SURFACE ULTRASTRUCTURAL CHANGES ON THIRD INSTAR LARVAE OF CHRYSOMYA ALBICEPS (DIPTERA : CALLIPHORIDAE) INDUCED BY CODEINE PHOSPHATE

BY

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ABSTRACT

The blowflies of Chrysomya albiceps are of medical and forensic importance. As they represent the most commonly studied insects in relation to forensic entomology because larvae of Chrysomya albiceps are the insects that are most commonly associated with corposes. The present work aimed at determination of the effect of codeine phosphate on Chrysomya albiceps larvae. The larvae in this work hatched from eggs of adult flies reared on rabbit tissues injected by lethal dose of codeine phosphate were used to observe the effect of the drug in comparison to control group. Both groups were observed by scanning electron microscopy (S.E.M.). Ultrastructural changes were observed in larvae reared on treated rabbits. These changes were in the form of distortion in cephalic segment where there were distortion in oral groove, antennal and maxillary sensory complex. There were marked swelling of the integment and tubercles specially on the caudal segment. There were marked affection on respiratory spiracles where anterior respiratory spiracles showed atrophy and obliteration of respiratory openings, while posterior respiratory spiracles showed hypogenesis and obliteration of respiratory slits by blebbing. These results indicate that codeine phosphate is capable of causing deformation in chrysomya albiceps larvae and that these larvae could be used in entomotoxicological purposes.

INTRODUCTION

Necrophagous flies particularly calliphorids, are recognized as the first wave of faunal succession on human cadavers (Wall and Warnes; 1994, Anderson, 2001; Amendt et al., 2004).

The blowfly, Chysomya albiceps is of medical and veterinary importance as myiasis producing flies and their involvement in the decomposition process. It is widely distributed throughout, Africa, South America and parts of Europe and Asia (Zumpt, 1965; Laurence, 1981; Po-

volny, 2002; Grassberger et al., 2003; Verves, 2004).

Usually, victims are not discovered for many days to months and, because of this, entomological techniques are useful in forensic analysis (Goff et al., 1992). Larvae are easily collected and maintained in the laboratory and they present less contaminant than tissues that are usually sampled for toxicological analysis (Goff and Lord, 1994). In addition, the study of larvae found in cadavers can also contribute to the quantitative identification of substances or abuse drugs present in the corpse (Kintz et al., 1990).

Codeine is a psychoactive alkaloid obtained from the opium poppy. It is specially used in therapeutics for its analgesic and antitussive properties (Maurer et al., 2006). Because it is an over - the - counter and psychoactive substance, it is often abused as substitute of heroine (Jensen and Hansen, 1993; Kintz et al., 1991). Codeine can be found in fatal cases as a result of drug intoxication after accidental or criminal administration.

Entomotoxicology is a relatively recent addition to the field of forensic entomology. Research into the effects of toxins on arthropod development is another major area of investigation in entomotoxicology, and is used to better estimate postmortem intervals (Entomotoxicology. http: // en.wikipedia.org).

This work was conducted in order to provide a basis for development a new method for determining the effect of drug such as codeine phosphate on the third instar larvae of chrysomya albiceps by Scanning Electron Microscopy (S.E.M.)

MATERIAL AND METHODS

Material :

1- Codeine phosphate powder was obtained from Knoll, USA.

2- Distilled water (for dissolving codeine phosphate and for control rabbits).

3- Four male domestic rabbits weighing 3.5 - 4 kg each were used as two controls and two treated with the lethal dose of codeine phosphate calculated according to Paget's formula (Paget and Barnes, 1964).

Methods :

* The experiment was run between 20th of June to 17th of July 2007.

* Two rabbits were injected with the lethal dose of codeine phosphate (28 mg/kg body weight dissolved in distilled water) and the other two rabbits used as control were injected with distilled water via ear vein. The four rab-

bits were sacrificed by cervical dislocation and were subsequently placed in cardboard boxes (60 x 60 x 40) floored with mudy soil and protected with metal cages with a 10 meter distance between the boxes of the two study groups and exposed in the roof of Assiut University Camb.

* The third instar larvae were collected from both treated and control groups and prepared for Scanning Electron Microscopy (S.E.M.).

* Scanning Electron Microscopy (S.E.M.) : Larvae were prepared for the SEM by fixing with 2.5% gluteraldehyde in phosphate buffer (pH 7.4) at 4°C for 24h. They were then rinsed with phosphate buffer (two times with 10 min interval). The rinsed larvae were post-fixed in 1% osmium telroxide at room temperature for 2-3 h. After rinsing two times with phosphate buffer, they were dehydrated in an increasing series of concentration of alcohol at 12 h intervals.

In the last step of dehydration, acetone was applied instead of absolute ethanol twice, with 30- min interval. The critical point drying was performed thereafter. These specimens were attached to brass holders, using carbon two component glue. They were coated with gold in a high- vacuum sputtering apparatus and stored in a desiccator until use (Collwell and O Connor, 2000). The specimens were observed with a JEOL.J S M 5400A Scanning Electron Microscopy (Japan).

Identification of larvae were carried out according to specific keys (Greenberg, 1971).

RESULTS

In this study codeine phosphate affect the third instar larvae of chrysomya albiceps as seen by S.E.M.

I- Anterior end:

In control larvae; normal appearance of the anterior end with normal hooks, normal oral grooves and normal sensory complex shown in Fig. (1). While in treated, chrysomya albiceps larvae exhibited deformed appearance of the anterior end (Fig. 2).

II- Anterior respiratory spiracles:-

When anterior spiracles were examined in control larvae it showed normal appearance where it had fan shaped and 10 respiratory opening (Fig. 3). While in treated larvae it showed fusion of respiratory spiracles at their root(Fig. 4) and deformed anterior spiracles with blebbing (Fig. 5). Also fusion and small sized spiracles (Fig. 6).

III- Posterior end:

The control showed normal appear-

ance, having normal shape and normal respiratory opening with normal surrounding area (Fig. 7). While in treated one the posterior end was deformed with abnormal processes (Fig. 8).

IV- Posterior respiratory spiracles:

Posterior spiracles showed normal shape (C shaped) centerally located with three respiratory openings(slits) (Fig. 9). In treated larvae posterior respiratory spiracles showed hypogenesis of spiracles (Fig. 10). At higher magnification normal respiratory spiracles were seen with normal slits (Fig. 11) in control larvae, and obliterated respiratory slits and bleb formation on there slits in treated larvae (Fig. 12).

DISCUSSION

In the setting of a suspicious death investigation, the toxicologist is very often solicited to find evidence of the presence of a psychotrophic and/or poisonous substances in the classic biologic liquid (blood, urine) in order to clarify the cause of death. However, these liquids alter with the deterioration of the corpse remnants. Although insect remains represent the main samples available for analysis after months or years, only few references deal with the potential toxicological interest of such samples (Bourel et al., 2001).

Necrophagous insect larvae are a living material which can be used to do toxicologic research (Mergaoui et al., 2007). There were studies dealing with the effects of different drugs or toxins on the development of many types of flies that were accelerate or retard their growth. For example codeine can stimulate the growth of L. sericata during the larval period (Kharbouche et al., 2007), larvae reared on rabbit tissues containing diazepam developed more rapidly than larvae from control (Carvalho et al., 2001). However, presence of morphine on the tissues accelerates the development of boettchersica peregrina and retards the growth rate of L. sericata during larval stage (Bourel et al., 1999; Kharbouche et al., 2007).

The present work has shown that codeine phosphate caused loss of architecture of the anterior end, and deformed anterior respiratory spiracles with blebbing or being fused and smaller in size. Deformed posterior end and posterior processes. Hypogenesis, obliterated and blebbing of the posterior respiratory spiracles of the treated third instar larvae of chrysomya albiceps when compared to the control by S.E.M.

There were two studies reported a possible association between cardiac and respiratory malformations and use of codeine during the first trimester of pregnancy (Briggs et al., 1986; Reynolds, 1989).

However, to our knowledge, this is the first report describing the effect of codeine on the third instar larvae of chrysomya albiceps by S.E.M. These results indicate that codeine phosphate is capable of causing deformation in chrysomya albiceps larvae and that these larvae could be used in entomotoxicological purposes.

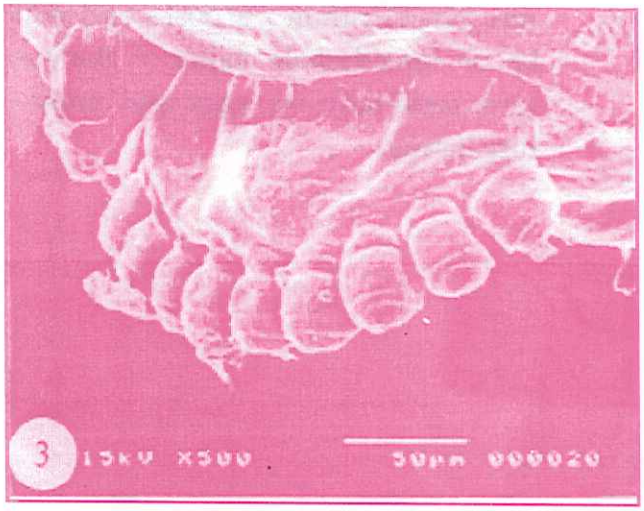
CONCLUSION

It is concluded that our study brings new elements of a considerable value for identification purposes and discovering the effects of codeine phosphate by using SEM. So when insects are found on the body, samples must be collected in conjunction with other biological evidence. Also specific collecting techniques and standard procedures in different environment at the death scene, at the autopsy and in the laboratory must be developed to enhance the recovery of insect specimens.

RECOMMENDATION

1- Encouragement of the interaction between forensic scientists involved in criminal investigations and in particular between pathologists and entomologists in death cases where insects are associated with the remains.

2- Scanning Electron Microscopy must be increasingly being used for routine identification purposes and will be of considerable value in the future, especially for eggs and immature larvae.



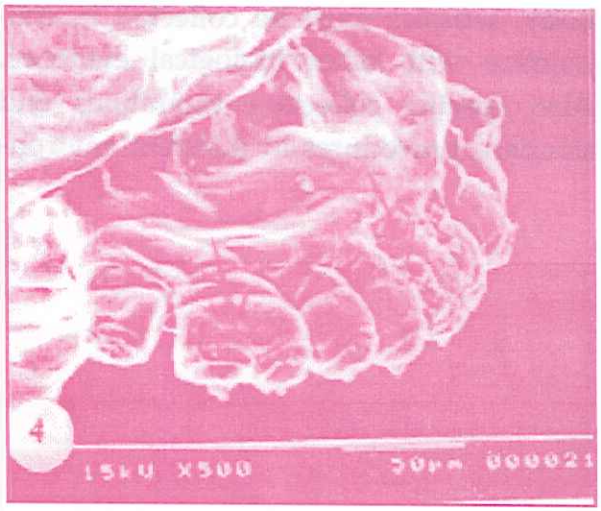
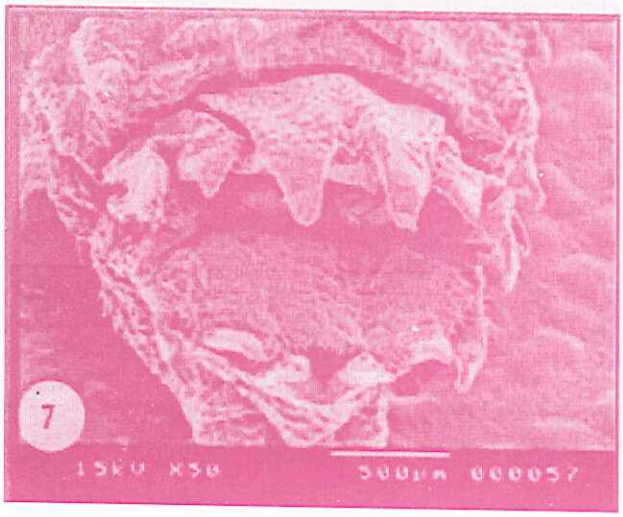


Fig. (1) : Control larvae anterior end showing normal mouth hooks, ridges and sensory complex.

Fig. (2) : Treated larvae anterior end showing loss of architecture and deformation.

Fig. (3) : Normal anterior respiratory spiracles.

Fig. (4) : Deformed anterior respiratory spiracles, two brancheae from one root (arrow), blebbing of one slit.



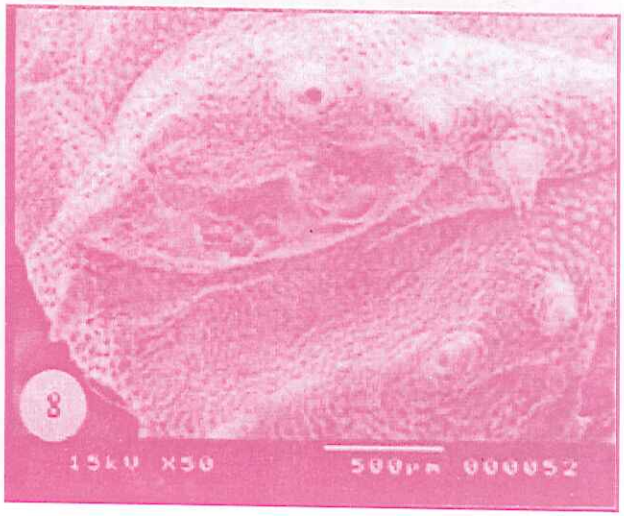
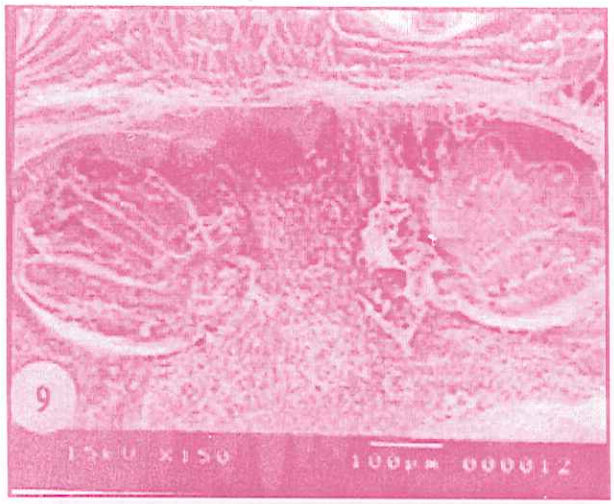
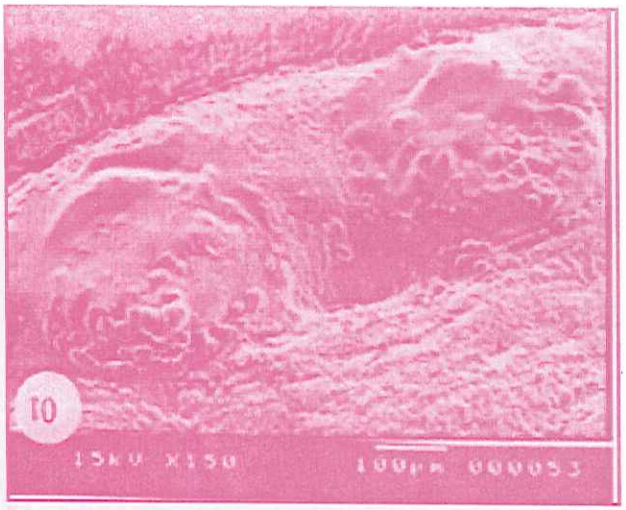


Fig. (5) : Deformed anterior spiracles with blebbing.
Fig. (6) : Deformed anterior spiracles being fused and smaller in size.
Fig. (7) : Normal posterior end and processes.
Fig. (8) : Deformed posterior end and processes.





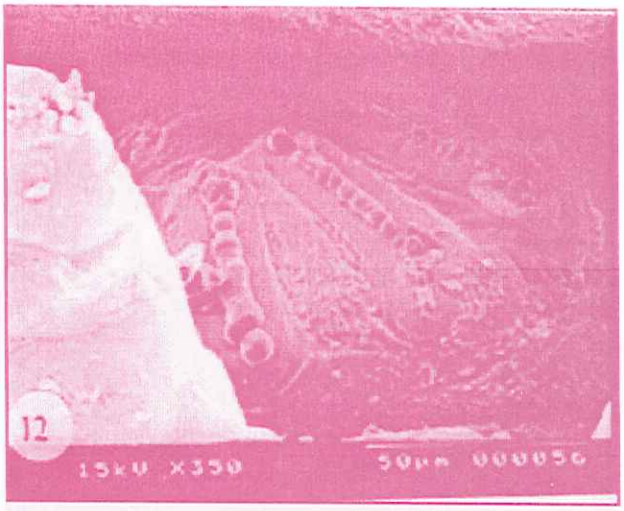


Fig. (9) : Normal respiratory spiracles.
Fig. (10) : Hypogenesis of posterior respiratory spiracles.
Fig. (11) : Normal posterior respiratory spiracles.
Fig. (12) : Obliterated posterior respiratory spiracles and blebbing.

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التغيرات فى التركيب السطحى الدقيق فى الطور اليرقى الثالث لذبابة الكريزومايا ألبيسبس (دابيترا : كاليفوريدى) نتيجة التسمم بالكودايين فوسفات

المشتركون فى البحث

د. مها سيد إبراهيم و د. هاله محمد فتحى*

من أقسام الطفيليات الطبية والطب الشرعى والسموم الإكلينيكية*

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تعتبر ذبابة الكريزومايا ألبيسبس ذات أهمية طبية وأهمية طبية شرعية لأنها تمثل واحدة من أكثر الأنواع التى تمت دراستها بالنسبة للحشرات التى لها أهمية طبية شرعية.

ويهدف هذا البحث إلى دراسة تأثير الجرعة المميتة من الكودايين فوسفات على يرقات ذبابة الكريزومايا ألبيسبس.

وقد تم الحصول على هذه اليرقات من بويضات الذبابة البالغة التى تم أخذها من أرانب تم حقنها بالجرعة المميتة بالكوادايين فوسفات وكذلك من الأرانب التى استعملت كمجموعة ضابطة.

وقد تم فحص المجموعتين بالميكروسكوب الإلكترونى الماسح وقد لوحظت تغيرات على سطح الجسم فى اليرقات المأخوذة من الأرانب التى تم حقنها بالكودايين وكانت هذه التغيرات على شكل تشوه فى الفجوات الفميه وقرون الاستشعار وأجزاء الفم وكذلك كان هناك إنتفاخ فى الطبقة الخارجية للجسم وبالذات فى الفقرة الأخيرة.

وأيضاً من الملاحظ أن هناك تأثير واضح على فتحات التنفس الأمامية حيث ظهر بها ضمور وانسدادات.

أما بالنسبة للفتحات التنفسية الخلفية فقد كان هناك ضعف فى تخليق الشكل النهائى وأحياناً إختفاء هذه الفتحات مع ظهور بروزان تسبب إنسداد بالفتحات الخلفية ومن هذا يمكننا أن نستنتج أن الكودايين فوسفات قادر على إحداث تشوهات فى يرقات الكريزومايا ألبيسبس ولهذا يمكن استعمال هذه اليرقات فى معرفة سبب الوفاة.