

METALDEHYDE INDUCING HISTOLOGICAL ALTERATIONS OF BROWN AND WHITE GARDEN SNAILS DIGESTIVE GLANDS

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ABSTRACT

The normal structure as well as any alterations or effects of both brown *Eobania vermiculata* and white *Theba pisana* garden snail hepatopancreatic cells (digestive gland) due to LC_0 (1/10 of the LC_{50} value) metaldehyde laboratory wheat bran bait treatments were investigated microscopically and recoded photographically. Histological studies showed distinct disturbed activity in the digestive gland tissues of both white and brown garden snails. The results revealed that: 1-Digestive glands of both tested snails were composed of multinumber of the digestive tubules with different type of cells in each tubule. 2- LC_0 of metaldehyde treatments induced inflammatory, ballon degeneration, thickining, fibrosis as well as focal manner of necrosed cells in both white and brown garden tested snails digestive cells.

INTRODUCTION

Metaldehyde – tetramized form of acetaldehyde - is one of the most common primary commercial successful molluscicide since 1937 and still being used against terrestrial snails (Lang and Macleod 1941; Gragg and Vincent 1952; Pappas and Carman 1955; Getzin 1965; Crowel 1967; Brar and Simwat 1973 and Abdallah *et al.*, 1998). Treatments of metaldehyde caused an excessive increase of fluid excretion in the form of foams around the soft snail body leading to snail death due to drop of body water content, that is very crucial for snail life activity (Abdallah *et al.*, 1992 and Kassem and Abdallah, 1992 and Kassem *et al.*, 1993). Bait formulations of molluscicides was the most effective application method in the field of terrestrial gastropods control programme rather than any other technique in this respect. Accordingly, the first target and direct site of bait formulated molluscicides action would be the cells of digestive gland as molluscicides are in use as oral toxins. (Triebskorn, 1989; Abdallah *et al.*, 1992 and Mohamed, 2003).

In the present study the normal structure as well as any alterations or effects of both brown (*Eobania vermiculata*) and white (*Theba pisana*) garden snails hepatopancreatic cells (digestive gland) due to metaldehyde treatments were investigated microscopically and recorded photographically as digestive gland (primary target organ for all oral molluscicides used) plays a central role in the detoxification as well as resorption of toxicants and food that land snail treated with or exposed to (Morton, 1983; Triebskorn, 1989; Bielefeld, 1991 and Mohamed, 2003).

MATERIALS AND METHODS

Two economically important terrestrial snails, *Theba pisana* and *Eobania vermiculata* were collected from El-Tarh citrus orchard farm, Beheira Governorate. Snails were classified according to Janus (1965) and Godan (1983) and separated in different enough glass jars and left at least 2 weeks under laboratory conditions before use.

Wheat bran baits with the appropriate concentration of metaldehyde were laboratory prepared according to Mohamed 2003. Five jars were used for each experiment (four replicates and one control). Ten animals were introduced in a marked jar covered with a transparent tissue and fixed with a rubber band. Water was sprayed daily on the covers to maintain humidity. Only untreated baits were used for the control. Samples of snails for each experiment were dissected after 7 days of the experiment. The procedure was as follows: the shell was broken gently by using small hammer, then the soft specimen was quickly dissected in a saline solution [Hedon Fleig's saline solution (Lee, 1950)]. The digestive gland was quickly isolated and transferred directly into 10% formalin, in order to overcome the problem of the immediate autolysis of the digestive gland. Dissection of the snail was carried out by the aid of binocular stereoscope for the removal of digestive gland. The isolated digestive glands were divided into suitable and marked portions to be convenient for routine histological preparation. The isolated organs were fixed for about 24-hours in 10% formalin. The fixed organs were dehydrated and xylene as a clearing agent. Embedding in paraffin wax with melting point 55-65°C that was followed. Sections were cut at 6-8 μ . The prepared sections were stained by using eosin-haematoxyline, or Mallory's triple stains. The stained sections were critically examined and some of the examined parts were photographed at different magnifications.

RESULTS AND DISCUSSION

The normal structure of heptopancreatic cell (digestive gland) and alterations due to LC₀ metaldehyde treatments were investigated.

The tubules of the digestive glands of the land snails brown garden snail *Eobania vermiculata* and white garden snail *Theba pisana* were carried out microscopically and photographically recorded before and after exposure to LC₀ of metaldehyde (Figures 1-8). The histological results of the examined digestive glands revealed that:

A: Histopathological findings of *Eobania vermiculata*:

1- Control group:

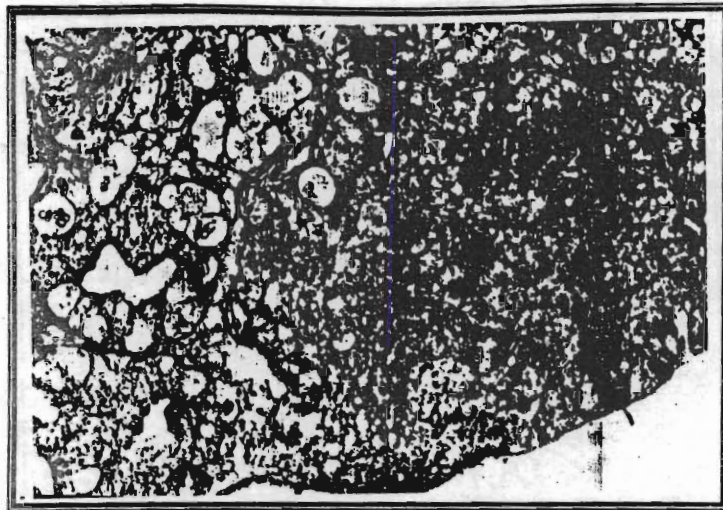
The digestive gland composed of multinumber of the digestive tubules there was different types of the cells in the tubules in which most of them were the digestive cells in digestive as well as excretory phases which had lipofuscin pigments and vacuoles. The second type of the cells in the digestive tubules was the basophilic cells which had calcium granules. The third cells were hardly identified and called thin cells (Fig.1 and 2).



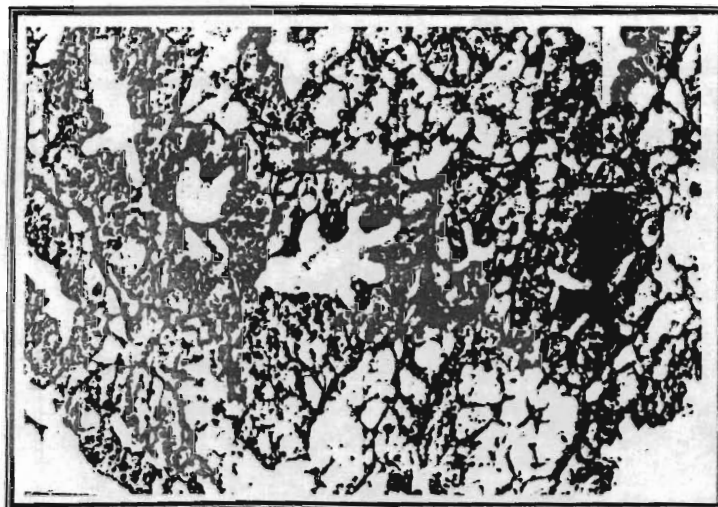
Fig(1): Digestive gland of *Eobania Vermiculata* in control group showing histological structure of the digestive tubules with various types of digestive cells (H&Ex40).



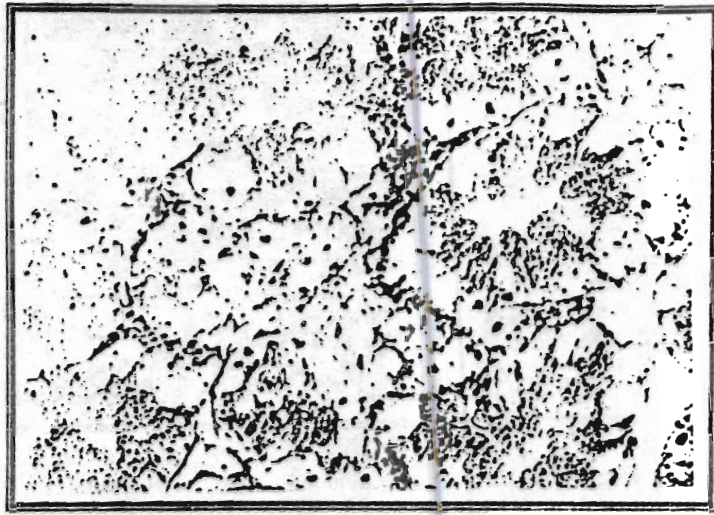
Fig(2): Digestive gland of *Eobania Vermiculata* in control group showing vacuoles in the digestive cells of excretory phase (H&Ex160)



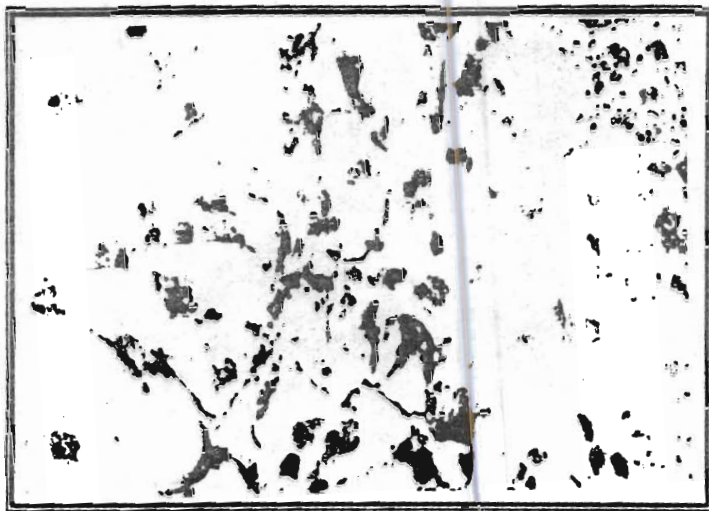
Fig(3): Digestive gland of *Eobania Vermiculata* treated by 1/10 LC₅₀ of metaldehyde for 10 days showing focal necrosed cell and necrtic clebris.(H&Ex40).



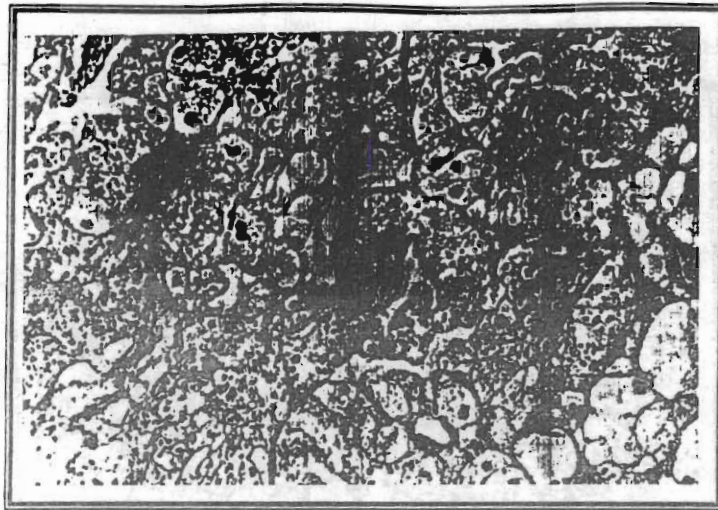
Fig(4): Digestive gland of *Eobania Vermiculata* treated by 1/10 LC₅₀ of metaldehyde for 10 days showing irregularitis of digestive tubules with ballon degeneration. (H&Ex40).



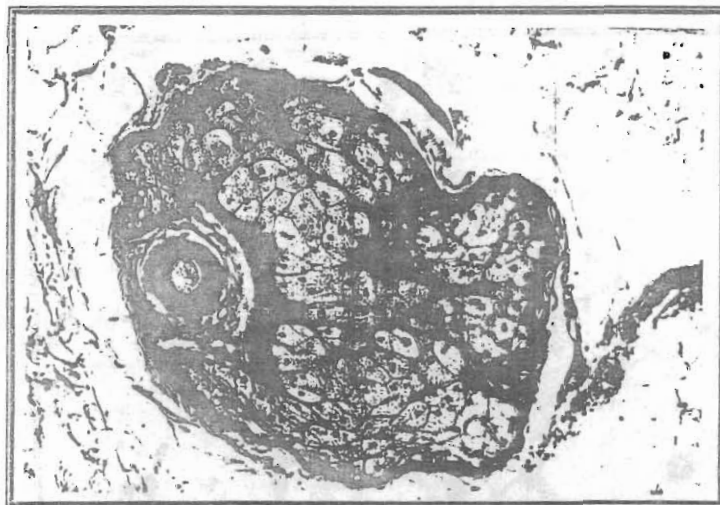
Fig(5): Digestive gland of *Theba pisana* in control group showing the histological structure of the tubules which gvae different types of cells. (H&Ex40).



Fig(6): Digestive gland of *Theba pisana* in control group showing basophilic calcium granules in the cytoplasm of the basophilic cells. (H&Ex160) .



Fig(7): Digestive gland of *Theba pisana* treated by $1/10$ LC_{50} of metaldehyde for 10 days showing ballon degeneration of the digestive cells surrounded by inflammatory cells mainly neutrophils. (H&Ex40).



Fig(8): Digestive gland of *Theba pisana* treated by $1/10$ LC_{50} of metaldehyde for 10 days showing hypertrophy of sebaceous like gland. (H&Ex40).

2- *E. vermiculata* treated by LC₀ of metaldehyde for 10 days:

Fig. 3 shows digestive glands surrounded by inflammatory cells mainly neutrophils in addition to mild balloon degeneration. Thickening, fibrosis and hypertrophy of sebaceous like gland (Fig. 4). The hypertrophy of such sebaceous cells might be partially responsible for increasing mucus secretion phenomena associated with metaldehyde treatment.

B: Histopathological findings of *Theba pisana*:

1- control group :

Histopathological examination of *T. pisana* digestive gland showed the same structure as *E. vermiculata* (Fig. 5&6).

2- *T. pisana* treated by LC₀ of metaldehyde for 10 days:

Focal manner of necrosed digestive cells was noticed as shown in Fig. 7. Irregularities of digestive tubes as well as balloon degeneration (Fig.8) was also observed due to metaldehyde treatments.

The role of digestive gland in the biotransformation and also as a direct site of baits molluscicidal actions is very important. In experiments on *Deroceras reticulatum* using radiolabelled cleothocarb and metaldehyde, radioactivity accumulated in the basophilic cells of the digestive gland a short time after application of chemicals (Triebkorn *et al.*, 1996) accompanied by increased activity of the phase I detoxification enzymes in the same cells, in addition to ultrastructural changes in the basophilic cells following the exposure to metaldehyde and carbamates (Triebkorn, 1989; Triebkorn and Künast, 1990 and Beshr, 2000). In addition, the ultrastructure of the hepatopencease of terrestrial gastropods reflects the impact of environmental pollutants as well as molluscicide exposure on the metabolic processes at the cellular level (Recio *et al.*, 1988; Triebkorn, 1989; Cajaraville *et al.*, 1990; Triebkorn and Künast, 1990 and Triebkorn, 1995). In our study, *in vivo* LC₀ ingestion using wheat bran bait laboratory formulated of metaldehyde induced different and remarkable ultrastructural changes and disturbed activity in the digestive gland cells of both *E. vermiculata* and *T. pisana* snails.

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التغيرات الهستولوجية لخلايا الغدد الهاضمة لكل من القوقعي الابيض والبنى نتيجة
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تم دراسة التركيب الطبيعي لخلايا الغدة الهاضمة فى القواقع الأرضية لكل من القوقع البنى (ايوبنيا فيرميكولوتا) و القوقع الأبيض (تيا بيسانانا) و كذلك التغيرات فى هذه الخلايا نتيجة المعاملة بطعم ردة القمح مع مبيد الميتالدهيد بقيمة LC_{50} (وهى ١٠/١ من LC_{50}) و فحصت هذه الخلايا ميكروسكوبيا كما تم تسجيلها بالصور.

أوضحت الدراسات الهستولوجية تأثيرات واضحة ومميزة فى أنسجة الغدد الهاضمة لكل من قواقع الحدائق الأبيض والبنى. ويمكن تلخيص النتائج فيما يلى : ١- الغدد الهاضمة لكل من القواقع المختبرة مركبة من العديد من القنوات الهاضمة الأنبيبية مع اختلاف نوع الخلايا فى كل قناة. ٢- معاملات الميتالدهيد بقيمة LC_{50} اظهرت تهيج وتحلل خلايا القنوات الأنبيبية تظهر فى صورة تجمعات فراغية وتغلظ و تليف وكذلك وجود شكل بؤرى غير منتشر من خلايا ميتة فى الخلايا الهاضمة لكل من قواقع الحدائق الأبيض و البنى المختبرة.