

Application of native excretory /secretory products from third larval instar of *Chrysomya megacephala* (Diptera:Calliphoridae) on an artificial wound

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Abstract: The analysis of native excretory/secretory products from third larval instar of *C.megacephala* using SDS-gel electrophoresis produces a band at 16KDa, a band between 16KDa and 23KDa and a broad band between 23 and 45KDa. This native excretory /secretory products cause the healing of an artificial wound in a rabbit. [Journal of American Science 2010;6(7):313-317]. (ISSN: 1545-1003).

.Key Words: *Chrysomya megacephala*, mid-gut, excretory/secretory products, maggot therapy

1. Introduction

Numerous clinical reports have been published that describe the outstanding effects of maggot therapy, most notably on debridement, cleansing, disinfection and healing of indolent wounds, many of which have previously failed to respond to conventional treatment. Current day maggot therapy, with its multi-action approach to wound cleansing and healing, is highly successful Sherman *et al.*, (2004) . Zasloff (2002) reported that antimicrobial peptides (AMPs) are an evolutionarily conserved component of the innate immune response, which is the principal defense system for the majority of living organisms, and are found among all classes of life ranging from prokaryotes to humans. Maier *et al.*, (1999) stated that they represent a new family of antibiotics that have stimulated research and clinical interest The benefits associated with the use of insect larvae or maggots in the healing of wounds were first observed and recognized centuries ago but it is only recently, especially with the increase in antibiotic resistant micro-organisms, that such use, termed "biosurgery", has been readdressed and the renaissance of maggot therapy begun (Sherman *et al.*, 2000). The larvae currently in clinical use are those of the green bottle fly *Lucilia sericata*. It is believed that larvae, or maggots, have a remarkable ability both to debride and to disinfect wounds. More recently it has been determined that this ability extends to the stimulation of tissue regeneration and to wound closure. Larvae are usable in the clinical setting to improve and to increase the rate of healing of many chronic wounds where conventional therapy such as antimicrobials,

surgical debridement and wound drainage are unable to reduce or to stop the progressive tissue destruction (Mumcuolgu *et al.*, 1999). Such chronic wounds originate from various conditions and include; diabetic foot ulcers, venous leg ulcers, infected surgical wounds, orthopedic wounds, osteomyelitis and pressure sores.

Despite of all these advantages of maggot debridement therapy (MDT), the utilization of MDT appears to be poor accepted by both patients and healthcare professionals. Social and cultural beliefs may initially hinder its use. There are other problems concerning the use of live maggots for MDT. Repeated changes are necessary because the life cycles of larvae are relatively short. Moreover, their limited shelf-life means that they need to be used soon after delivery. The most important problem is rearing these maggots in hospitals without smelling their bad odor, which is impossible even with very high sanitary conditions (Dominic *et al.*, 2007)

The constituents of the larval excretory/secretory (ES) products are thought to be central to the way in which maggots promote wound healing with several mechanisms proposed to explain their actions. Debridement is thought to be partially achieved via the proteolytic action of collagenase and serine proteases present in the ES which degrade the necrotic tissue into a form which is ingestible to the larvae and which act to remove the slough from the wound surface (Beasley *et al.*, 2004).

The stimulation of extra-cellular matrix (ECM) remodeling and closure has also been proposed to be attributable to the action of one or

more 'chymotrypsin-like,' serine protease/s present in the ES which acts to degrade fibronectin into bioactive peptides. It is thought that these bioactive peptides are then able to stimulate adhesion and migration of fibroblasts (Horobin *et al.*,2005)and are possibly responsible for the acceleration in healing(Horobin *et al.*,2003).The ES may also have an effect on fibroblast proliferation.

Chrysomya megacephala (F.), the Oriental latrine fly, is a common blow fly species of medical importance in many parts of the world, including Egypt. Adults may feed on food sources including nectar, animal carcasses, garbage, and other filth materials, or even human food. Therefore, it is possible that mechanical transfer of potential disease causing pathogens, such as bacteria, viruses, protozoa, and helminth eggs, to human food may occur (Sukontason *et al.*, 2000). Larvae of this species are known to cause myiasis in several mammal species, including humans (Kumarasinghe *et al.*, 2000). Another facet of medical importance of this blow fly is its association with human corpses and its relevance to forensic entomology, *C. megacephala* were found connected with cases of human death (Sukontason *et al.*, 2005).

The aim of this paper,was to check the healing action of native excretory/secretory products from third larval instar of *Chrysomya megacephala* on a wound induced in a rabbit.

2. Material and Methods

The laboratory colony of *C. megacephala* used in this study was established in the Department of Entomology, Faculty of Science, Helwan University. *Chrysomya megacephala* was reared according to the method of Gaber *et al.*, (2005). Native excretions/secretions (nES) were collected by incubating third-instar larvae of *C. megacephala* in a small quantity(100 larvae per 1ml) of sterile distilled water for 1 h at 30 °C in darkness. The sterile liquid was siphoned from the containers and centrifuged at $10,000 \times g$ for 5 min to remove particulate material, after which the supernatant was stored at 4°C for testing.

Male rabbit about 1.5 Kg weight,was used for this experiment.-Rabbit was kept in a big wooden box with wire.Water and food were daily given to the rabbit.The rabbit was partially anthesized. The upper thigh of the rabbit was shaved before induction of wound.

A drop of sulphuric acid was added to the skin to induce a severe wound.Photographs of the wound were taken with a digital camera 10 pixels.-The native excretory /secretory(nES) products was

prepared with concentration 100 larvae per 1 ml distilled water.3-5ml nES was applied daily on the wound.-Daily observation of the changes in the wound were recorded and photographed.

3. Results

The analysis of native excretory/secretory products from third larval instar of *Chrysomya megacephala* produces a single band at 16KDa,a band between 16KDa and 23KDa and a broad band between 23KDa and 45KDa (Fig. 1). The tissue of the wound induced in the rabbit appeared to be dead (Fig.2).By applying the nE/S daily the tissue appears to be more healthy(Figs 3,4,5).Flow of blood can be seen and also the formation of collagen (Fig6).

4. Discussion

Proteolytic enzymes are a major component of the digestive process of parasites and are presumed to be released to interact with host tissues (Rhoads and Fetterer, 1997).

The analysis of native excretory/secretory products from third larval instar of *Chrysomya megacephala* produces a single band at 16KDa and a broad band between 23KDa and 45KDa. Insect trypsin have been characterized and purified from species of Coleoptera, Orthoptera, Lepidoptera and Diptera. Most insect trypsin are 20–30 kDa as determined by SDS-PAGE (Terra and Ferreira, 1994). These insect trypsin are most active at alkaline pH, are not activated by calcium ions, and are sensitive to natural trypsin inhibitors (Terra and Ferreira, 1994). Recently, workers in Nottingham, UK, demonstrated *in vitro* a range of enzymes secreted by *P. sericata* larvae (Chambers *et al.*,2003).

Four proteolytic enzymes, comprising two serine proteases, a metalloproteinase and an aspartyl proteinase, were detected, with molecular weights ranging from 20 to 40 kDa, with activity across a wide pH range. It is clear from the results of the present study that the wound healed in the rabbit may be due to the action of the proteolytic enzymes present in excretory/secretory products from third larval instars of *C. megacephala*. Research into the debridement mechanisms underlying maggot therapy has revealed that maggots secrete a rich soup of digestive enzymes while feeding, including carboxypeptidases A and B ,leucine aminopeptidase (Vistnes *et al.*,1981), collagenase (Ziffern *et al.*,1953) and serine proteases (trypsin-like and chymotrypsin-like enzymes) (Casu,1994).

Enzymes can be produced from any living organism, either by extracting them from their cells or by recovering them from cell exudates (Lambert

and Meers 1983). The molecules involved in the beneficial effects of maggots are believed to be contained in their excretions/secretions (ES) (Mariena *et al.*,2007). Britland (2006) established the wound-healing capacity of "maggot juice" by applying extracts of the secretion to layers of cells that mimic skin. When they created artificial, circular "wounds" in the layers, the wounds healed fastest when exposed to the extracts. They suggest that protease enzymes in the juice enable repair cells to move more swiftly and freely within the wound site. "They all march in unison and fill the hole significantly quicker," says co-team leader, David Pritchard at the University of Nottingham in the UK. The researchers showed that the holes healed just as quickly whether the juice was applied directly or in a

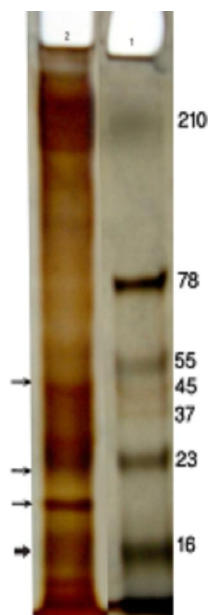
prototype gel which could be developed into a wound dressing.

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Fig(1):Electrophoretic pattern using SDS-gel electrophoresis.Lane(1) representing marker and lane(2) representing native excretory/secretory product produced from third larval instars of *Chrysomya megacepha*



Fig (2) The wound after adding sulphuric acid the tissue becomes dead.



Fig (3) The wound after one day from adding nES, the tissue starts healing.



Fig (4) The wound after 3 days from adding nES, the tissue continues healing.

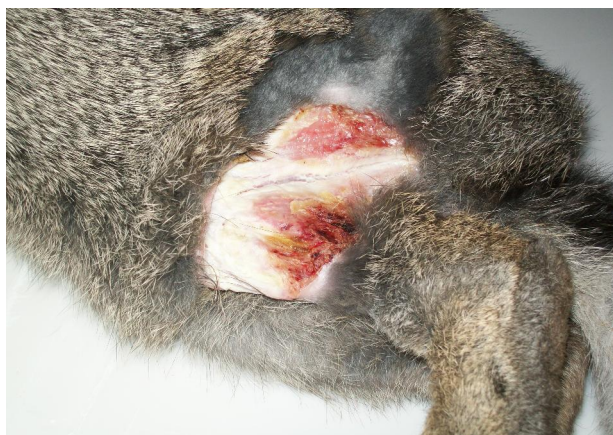


Fig (5) The wound after 5 days from adding nES, the tissue.



Fig (6): The wound after 7 days from adding n ES, the tissue starts healing.

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