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The anti-bacterial of whole body homogenates of third instar larvae of *Chrysomya megacephala* (Diptera:Calliphoridae) against different bacterial strains

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ABSTRACT

The present study evaluates the potential anti-bacterial activity of homogenates of the whole body larvae of third instars' larvae of *Chrysomya megacephala* against gram -ve bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and gram +ve bacteria (*Staphylococus aureus, Bacillis subtilis*). The homogenates of the whole body larvae showed antibacterial activity against *E.coli, S. aureus, B. subtilis.* The minimum inhibitory concentrations (MIC) were 125ug/ml, 31.25ug/ml,15.63ug/ml, respectively. The homogenates of the third instars larvae of *Chrysomya megacephala* have no effect on *Pseudomonas aeruginosa.*

Keywords:, *Chrysomya megacephala,* antibacterial effect.

INTRODUCTION

Many dipteran species are capable of infesting living vertebrate hosts (a condition termed myiasis). Maggot therapy is essentially artificially induced myiasis, performed in a controlled environment by experienced medical practitioners. Maggot therapy has the following three beneficial effects on a wound: debridement, disinfection and enhanced healing. 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 (Ziffren *et al*.,1953) and serine proteases (trypsin-like and chymotrypsin-like enzymes) (Casu *et al*.,1994). The majority of wounds are polymicrobial, hosting a range of both anaerobic and aerobic bacteria (Bowler and Davies, 1999). Antimicrobial treatment of clinically infected and non-healing wounds, should, therefore, encompass broad-spectrum antimicrobials in order to cleanse the wound effectively. The application of maggots to an infected wound results in the rapid elimination of such infecting microorganisms (Courtenay 1999). The most frequently isolated pathogen from acute and chronic wounds is *Staphylococcus aureus*.

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 helminthes eggs, to human food may occur (Sukontason, 2000). Larvae of this species are known to cause myiasis in several mammal species, including humans (Kumarasinghe, 2000). Another facet of medical importance of this blow fly is its association with human corpses and its relevance to forensic entomology. Many researchers have reported that specimens of *C. megacephala* were found connected with cases of human death (Sukontason, 2005).

The aim of the present study is to investigate the antibacterial effect of homogenates of the whole larval bodies against some bacterial strains.

MATERIALS AND METHODS

1- Rearing of insect:

The laboratory colony of *C. megacephala* used in this study was established in the Department of Entomology, Faculty of Science, Helwan University. *C. megacephala* was reared following the reported protocol (Gabre *et al*., 2005). They were identified according to the mentioned method (Zumpt, 1965). Adults from the stock colony of *C. megacephala* were kept in cages (38×38×56 cm) at 25±3°C, 14h photoperiod and 60–70% R.H. The cages were made with a wooden floor, a glass roof, and wire gauze on three of the sides. The fourth side was wooden with a circular hole fitted with a cloth sleeve to facilitate daily feeding, cleaning of the cage, and removal of eggs. Adults were supplied daily with granular sucrose, water, and pieces of liver.

Water was supplied by dipping a piece of cotton as a wick in a bottle filled with water, and the liver was provided in a Petri- dish. Egg batches were removed daily and transferred to a fresh piece of chicken placed in a rearing enamel bowl (35 cm in diameter) covered with muslin secured with a rubber band. At the prepupal stage, dry autoclaved sawdust was added to the bowl as a medium for pupation. Pupae were sieved from the sawdust and transferred to adult cages described above for adult emergence.

2- Preparation of crude extracts from insect larvae:

Preparation of larval homogenate:

The whole third instar of insect larvae were (20,000 larvae in 500 ml) homogenized in distilled water and centrifuged, the supernatant was collected and separated .Part of it was stored at 4c for enzyme assay and the other part was lypophilized for antibacterial tests.

3- Antimicrobial assay:

3.1. Agar well diffusion method

The antibacterial activity of synthesized compounds was determined using agar well diffusion method (Scott, 1989). All the compounds were tested *in vitro* for their antibacterial activity against Gram positive bacteria (*Staphylococcus aureus Bacillus substilis*, and Gram negative bacteria (*Escherichia coli* , *Pseudomonas aeruginosa)* using nutrient agar medium. Ampicillin and gentamycine were used as standard drugs for Gram positive, Gram negative activity respectively. DMSO was used as solvent control. The compounds were tested at a concentration of 1 mg/ml against bacterial strains.

The sterilized media was poured onto the sterilized Petri dishes (20-25 ml, each petri dish) and allowed to solidify. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media and solutions of the test compounds were added to each well with the help of micropipette. The plates were incubated at 37°C for 24 hrs for antibacterial activity. This experiment was carried out in triplicate and zones of inhibition were measured in mm. scale.

3.2 Determination of minimum inhibitory concentration (MIC)

The MIC was determined by the broth microdilution method using 96-well micro-plates (Saini *et al.*, 2005; Bhuiyan *et al.*, 2011). The inoculate of the microbial strains was prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (1.0 mg) was dissolved in DMSO (1 mL) to obtain 1000 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to the well from row B to H. The stock solutions of samples (100 µL) were added to the wells in rows A and B. Then, the mixture of samples and sterile broth (100 µL) in row B was transferred to each well in order to obtain a twofold serial

dilution of the stock samples (concentration of 500, 250, 125, 62.5, 31.3, 15.6 and 7.81,3.9,1.95, 0.98 and 0.49 µg/mL). The inoculums (100 µL) were added to each well and a final volume 200 µL was obtained in each well. Plates were incubated t 37°C for 24 hrs for antibacterial activity. Bacterial growth was indicated by the presence of turbidity and a pellet at the bottom of the well.

RESULTS

The present study also evaluate the potential anti-bacterial activity of homogenates of whole body larvae of third instars' larvae of *C. megacephala* against gram -ve bacteria (*Escherichia coli,Pseudomonas aeruginosa*),gram +ve bacteria (*Staphylococus aureus, Bacillis subtilis*).

The homogenates of whole body larvae showed antimicrobial activity against *Escherichia coli, Staphylococus aureus, Bacillis subtilis.* The minimum inhibitory concentrations (MIC) was 125ug/ml,31.25ug/ml,15.63ug/ml, respectively. The homogenates of third instar larvae of *C. megacephala* have no effect on *Pseudomonas aeruginosa.*

Table (1): Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on gram positive bacteria using (1mg/ml) concentration of tested samples. Minimum inhibitory concentration (MIC) was determined.

Tested micro-organisms (Gram positive bacteria)	**Homogenates of whole larvae**	**MIC µg**	**Standard *Ampicillin***
***Staphylococcus aureus* (RCMB 010028)**	18.1±0.35	31.25	27.4+±0.18
***Bacillis subtilis* (RCMB 010067)**	19.4±0.64	15.63	32.4±0.10

Table (2): Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on gram negative bacteria using (1mg/ml) concentration of tested samples. Minimum inhibitory concentration (MIC) was determined.NA expressing no activity.

Tested micro-organisms (Gram negative bacteria)	**Homogenates of whole larvae**	**MIC µg**	**Standard *Gentamicin***
***Pseudomonas aeruginosa* (RCMB 010043)**	NA	NA	17.3±0.15
***Escherichia coli* (RCMB 010052)**	14.0±0.64	125	22.3±0.18

DISSCUSSION

The occurrence of different digestive enzymes in insects is frequently said to depend mainly on the chemical composition of the diet ingested by the animals (Wigglesworth, 1965).Enzymes responsible for the complete hydrolysis of proteins down to amino acids are the proteases. Proteases are enzymes acting on peptide bonds and include exopeptidases and endopeptidases. Endopeptidases are divided into sub-classes on the basis of catalytic mechanism. Serine proteases are endopeptidases and have a serine and a histidine in the active site (Terra and Ferreira, 1994). The serine proteases (SP) are the dominant class of proteolytic enzymes in many insect species (Terra *et al*., 1996). SP carry out a diverse array of physiological functions, the best known being digestion, blood clotting, fibrinolysis, fertilization, and complement activation during immune responses (Horl, 1989). They have also been shown to be associated with many diseases including cancer, arthritis, and emphysema (Diamandis and Yousef, 2002).The excreted/secreted serine proteases of *Lucilia cuprina* (sheep blowfly) larvae are thought to be involved in wound formation, and the provision of nutrients to the feeding larvae (Young *et al*.,1996). Similar roles have been

established for the same group of proteases secreted by *Chrysomya bezziana* (Muharsini *et al*., 2001).

The present study also evaluate the potential anti-bacterial activity of homogenates of whole body larvae of third instars' larvae of *C. megacephala* against gram -ve bacteria (*Escherichia coli,Pseudomonas aeruginosa*),gram +ve bacteria (*Staphylococus aureus,Bacillis subtilis*)

The homogenates of whole body larvae showed antimicrobial activity against *Escherichia coli, Staphylococus aureus,Bacillis subtilis,*.The minimum inhibitory concentrations (MIC) was 125ug/ml,31.25ug/ml,15.63ug/ml, respectively. The homogenates of third instar larvae of *C.megacephala* have no effect on *Pseudomonas aeruginosa*.

Several published studies suggest possible anti-bacterial properties of maggots and their secretions as an explanation for the success observed in the clinic [Daeschlein et al.,2007].It was reported by many authors (Moch *et al*., 1999) the mechanism of action of maggot disinfection on wounds and they found that the excretion of maggots exhibited a strong and rapid disinfection action on *S. aureus.* Kerridge *et al*. (2005) performed a zone of inhibition assay showing anti-bacterial activity of native excretory/secretory product against gram-positive bacteria such as *S. aureus and Streptococcus pyogenes*, whereas Bexfield *et al.* (2004), using a similar method and found no anti-bacterial activity.Similarly, antibacterial activity against both gram-positive and gram-negative bacteria including *S aureus and E. coli* has further been documented but whole body extracts and the haemolymph were used instead of of ES (Huberman, 2007a).The whole body extracts and haemolymph fractions from maggots lysed gram positive and gram negative bacteria including *P. aeruginosa, Klebsiella pneumonia and MRSA* isolated from wound.

During feeding, maggots produce a cocktail of proteolytic and anti-microbial substances called ES products of the gut as well as salivary glands origin.In vitro examination of ES products revealed substances including serine proteases chymotrypsin and trypsin –like protease (Thomas *et al*., 1999).

In general, the exact mechanism and components of the maggot's antibacterial activity are still unknown. The action of maggots can increase the microcirculation and probably destroy the very complex structure of a biofilm, consequentially the bacteria become susceptible to actions of antibiotics and the immune system as well as to actions of maggots (Mumcuoglu *et al*., 2001). On the other hand, it can be expected that several different antibacterial components, like oligopeptides, disinfectants and low pH act synergistically (Bexfield *et al*., 2004). Thus, it could be concluded that the different effects of maggot on Gram positive and Gram negative bacteria are mediated by different molecules and mechanisms (Van der Plas *et al*., 2008). *Proteus* spp. can colonize maggots as well as it is one of the maggots' gut commensals. It is noteworthy that *Providencia rettgeri* (previously known as *Proteus rettgeri*) produces an L-amino acid oxidase which could act on the larval antibacterial oligopeptides. Thus, the persistence of *Proteus* spp. after maggot applications in together with the survival of such organism in wound myiatic cases from which *Lucilia sericata* was isolated may be explained by the little effect of maggots on this bacteria due to either bacterial adaptation,or their symbiotic relationships as members of the *Lucilia sericata* gut flora (Jaklic *et al*.,2008). The failure of MDT in complete eradication of *Pseudomonas* may be related to the biofilm formation (Van der Plas *et al*., 2008) noticed that more ES required to disrupt *P. aeruginosa* biofilms than *S. aureus* biofilms. In addition, it has been shown in vitro that *P. aeruginosa*, but not *S. aureus*, impairs maggot survival .Together, these data are in agreement with clinical findings indicating that more maggots should be used for wounds infected with *P. aeruginosa* (Steenvoorde and Jukema, 2004).

Conclusion :

The present study revealed the antibacterial effect of whole homognates of third instar larvae of *C. megacephala* on different bacterial strains. These results support the need for further experiments aimed at validating *C. megacephala* use in larval therapy.

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The anti-bacterial of whole body homogenates of third instar larvae of *Chrysomya megacephala* (Diptera:Calliphoridae) against different bacterial strains

مضاد للبكتيريا لكامل الجسم متجانس ليرقات الطور الثالث من *Chrysomya megacephala*
(Diptera: Calliphoridae) ضد السلالات البكتيرية المختلفة

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المستخلص

الدراسة الحالية قيمت النشاط المضاد للبكتيريا المحتمل لمستخلص يرقات الجسم كله ليرقات الأطوار الثالثة من *Chrysomya megacephala* ضد بكتيريا الجرام (*Escherichia coli*، *Pseudomonas aeruginosa*) وبكتيريا gram + ve (Staphylococus aureus، *Bacillis subtilis*). أظهر مستخلص يرقات الجسم كله نشاطًا مضادًا للبكتيريا *Escherichia coli* و *Staphylococus aureus* و *Bacillis subtilis* وكانت التركيزات المثبطة الأدنى (MIC) 125 ميكروغرام / مل ، 31.25 ميكروغرام / مل ، 15.63 ميكروغرام / مل على التوالي. لم يكن لمستخلص يرقات العمر الثالث من *Chrysomya megacephala* أي تأثير على *Pseudomonas aeruginosa*.