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Research Paper

STUDIES ON HISTOPATHOLOGICAL EFFECTS OF DELTAMETHRIN ON THE MIDGUT OF ORIENTAL LATRINE FLY, *Chrysomya megacephala* (FABRICIUS) (DIPTERA: CALLIPHORIDAE)

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Abstract

The alimentary canal is a major organ system that is often involved in the transmission of pathogens to humans from insects that serve as vectors of disease. *Chrysomya megacephala*, is a medically important blow fly species. Adults have been implicated as mechanical carriers of numerous pathogens, such as bacteria, viruses, protozoa, and helminth eggs, which may cause diseases (e.g. diarrhoea, gastroenteritis, ulcers, nosocomial infections, cholera and dysentery) in humans, whereas larvae have been recorded as myiasis-producing agents. Another facet of medical importance of this fly is its association with human corpses and its relevance to forensic entomology. This study aimed to investigate the midgut of adult *C. megacephala* at the ultrastructural level using light microscopy. Insecticide deltamethrin of the concentration 0.05% & 0.005% was provided in food to 5th day adult flies to examine the alteration from normal histology of midgut. Midgut is an important part of alimentary canal because most of the digestion takes place in it. In midgut, flies treated with 0.05% concentration showed a significant enlargement in epithelium cells and lumen of the midgut decreased. In middle and posterior midgut, the epithelial cells were completely ruptured and separated from the basement membrane. The peritrophic membrane was not closely lying to the epithelial cells. Flies treated with 0.005% concentration showed degeneration and distortion in the shape of epithelial cells, but peritrophic membrane was less degenerated. Outer muscular layer showed no changes indicating no damage in both concentrations of insecticide.

Key words: *Chrysomya megacephala*, midgut, deltamethrin, histopathology, ultrastructure.

INTRODUCTION

*Chrysomya megacephala,*the oriental latrine fly, is commonly found in cadavers in many parts of the world [Gruner *et al*., 2007; Sukontason *et al*., 2007; Wang *et al*., 2008] and is used in forensic entomology cases, for postmortem interval determination [Goff & Odum, 1987; Goff *et al*., 1988; Goff, 1992]. Insects are useful sources for toxicological analysis when the tissues or body fluids normally used are not available. The study of insects, especially larvae found in cadavers, can contribute to the qualitative

identification of substances or illegal drugs present in the corpse [Nolte *et al.,* 1992; Kintz *et al*., 1990; Introna *et al.,* 1990]. In addition, drugs in putrefied tissues may have an influence on the development of the necrophagous Diptera that can affect the estimation of the PMI [Goff *et al*., 1991, 1992, 1993; Bourel *et al.,* 1999; Carvalho *et al*., 2001; O'Brien and Turner, 2004].

Chrysomya megacephala (Fabricius), is a member of the family calliphoridae and is a medically important blow fly species. It is a warm-weather fly with a greenish-blue metallic box-like body. *C. megacephala* is distributed widely over continents worldwide, extending from Oriental Asia, Australasia, Africa, Europe and the Mediterranean to North and South America [Boonsriwong *et al.,* 2012]. Adults feed on food sources including nectar, animal carcasses, and other filth materials, or even human food [Boonsriwong *et al.,* 2007]. Adults have been implicated as mechanical carriers of numerous pathogens, including bacteria, viruses, and parasites, that may cause human disease, whereas larvae have been recorded as myiasis producing agents [Zumpt, 1965]. Myiasis is infestation by the larvae (maggots) of fly species within the arthropod order Diptera. The larvae feed on the host's dead or living tissue, body substances, or ingested food. Myiasis can be categorised clinically based on the area of the body infested, for example cutaneous, ophthalmic, auricular and urogenital. Based on the close association of *C. megacephala* with humans and/or animals, which may be either disadvantageous or desirable from a forensic entomology viewpoint, diverse biological knowledge pertaining to this fly is essential in order to manage it. Few works on the alimentary canal of *C. megacephala* have been conducted; therefore, much information is lacking [Boonsriwong *et al*., 2007]. Despite the abundance and medical significance of this species, the lack of information on its digestive system led to this study, which was to observe the effects of insecticide deltamethrin of concentrations 0.05% & 0.005% on the gut of 5th day adult *C. megacephala* fly. Deltamethrin is a member of one of the safest classes of pesticides: synthetic pyrethroids. Exposure to low doses of insecticides has affected the gut in larvae of *Culex pipiens* by water extract of *Eichhornia crassipes* [Assar and El- Sobky, 2003], in *Blattella germanica* treated with boric acid [Habes *et al*., 2006], in *Mythimna separata* treated with fraxinellone [Lu *et al*., 2010], in *Spodoptera littoralis* by the action of pyridalyl [Dahi *et al*., 2011], in *Periplaneta americana* treated with *Datura alba* leaf extract [Khan *et al*., 2011] and with N-nitroso-N-methylurea [Jain & Ahi, 2014], in *C. megacephala* treated with malathion [Bakr *et al*., 2012], in Red Palm Weevil *Rhynchophorus ferrugineus* treated with Zinc Sulfate [Al-Dhafar and Sharaby, 2012].

MATERIALS AND METHODS

Rearing and Maintenance of *Chrysomya megacephala:* The adults of blow fly were collected from the Aligarh Muslim University campus by using rotten buffalo meat and brought to the laboratory for present research work. These fly colonies were maintained in rearing cages which are made up of wood and wire mesh and the cages were placed in incubator maintained at 27±2°C temperature and 60±5% relative humidity. The flies were fed on the mixture of sugar, protein and milk in the ratio of 1:1:3 soaked in cotton, which were changed daily. Chopped buffalo meat was provided as egg laying medium. When the eggs were laid, they were transferred to fresh rearing jars to ward-off any hindrance during egg-hatching. Over-crowding of larvae in jar was avoided for proper development of insect. Cotton was put in the jar at 3rd instar larval stage for pupation. Pupae were transferred to fresh jar for adult emergence.

Sampling of experimental insects: In the present work, 5th day adult flies were treated with 0.05% and 0.005% concentrations of insecticide deltamethrin by ingestion

method. After 24 hours of treatment, approximately 10 specimens of each concentration were removed from the rearing box and individually dissected in petridish containing Ringer's solution to remove alimentary canal by using fine entomological needles under a binocular dissecting microscope at 40X magnification and transferred to cavity blocks.

Histological preparation of midgut for light microscopy

The midgut was fixed immediately in Bouin's solution for 18 hours, transferred to distilled water (2 changes) for 15 minutes each and then dehydrated in ascending grades of alcohol 30%, 50%, 70%, for 15 minutes each, then stained in aqueous eosin for 2 minutes and then in 80% and 90% for 15 minutes each while in 96% and 100% for half an hour each followed by xylene for 2 minutes. Incubation was done at 63°C in xylene and parrafin wax (1:1) for 15 minutes and then in pure parrafin wax for 1 hour. A part of midgut was then embedded in parrafin wax whose 5 µm sections were cut into rolling ribbon. The ribbon was placed on the glass slide which was lubricated by egg albumin having few drops of glycerine. The slides containing section were warmed slightly to straighten the creases and then they were processed in xylene (2 changes) for 10 minutes each, followed by descending grades of alcohol 100%, 96%, 90%, 80%, 70%, 50%, 30% and then in distilled water for 5 minutes each. The slides were then stained in delafield's haematoxylin for 20 seconds and then processed in tap water & distilled water. After dehydration upto 70% alcohol, slides were counterstained with alcoholic eosin for 20 minutes and then dehydrated in alcohol series up to 100% for 5 min each. The slides were then kept in xylene (2 changes) for 10 min each, finally mounted with DPX and observed under compound light microscope. The histological preparation of control midgut was also done to compare the effects. Photomicrographs were taken using LEICA compound microscope using appropriate magnification.

RESULTS AND DISCUSSION

Normal histology of *Chrysomya megacephala*

The gut of *C. megacephala* consists of foregut, midgut and hindgut with sphincters controlling food movement between regions. The foregut is subdivided into a pharynx, an esophagus, a crop (food storage area) and anterior portion of the cardia with the salivary glands. At the anterior end of the foregut, the mouth opens into a preoral cavity which leads through pharynx into tubular esophagus. The crop laterally diverges from the esophagus as an extremely enlarged diverticulum. Posterior to the tubular esophagus is the bulb-like cardia. This structure is composed of two parts: the anterior foregut tissue and posterior midgut tissue. Thus, cardia comprises a junction of the foregut and midgut.

The fly midgut of *C. megacephala* is the longest portion of the alimentary canal lying convoluted and twisted within the body cavity. Midgut is further subdivided into the anterior, middle and posterior midgut. The anterior midgut emerges from the cardia and junction of the gastric caeca. Posterior to the anterior midgut is the more dilated area, the middle midgut. The anterior and middle segments are comparatively thick and the posterior segment becoming narrow and convoluted at the hind end.

The midgut proper begins with the posterior midgut tissue of the cardia just anterior to the gastric caeca. Four long tubular gastric caeca were observed in this species. The external surface of midgut is very smooth with inner circular and outer longitudinal muscles within. Insertion of tracheal tubules into the midgut surface is also quite prevalent in this area. Midgut tissue contains peritrophic membrane (PM) within its central lumen and is surrounded by a single layer of cuboidal epithelial cells. The peritrophic membrane present as a thin, transparent membrane. Epithelial cells project

inward from their basement membranes. The thickness of the basement membrane in *C.megacephala* depends on nutrition during the larval stage which facilitates the transport of products between the intestine and the haemolymph.
Typically, the beginning of the hindgut is defined by the entry point of the Malpighian tubules into a distinct pylorus forming into a muscular pyloric sphincter, followed by the ileum, colon, rectum and posterior anus. Both the foregut and hindgut have a cuticular lining, whereas the midgut does not.

Flies treated with deltamethrin: In anterior midgut, at 0.05% concentration treated flies showed a significant enlargement in epithelium cells and lumen of the midgut decreased and an identical result was obtained in *Blatella germanica* treated with boric acid by Habes *et al*. (2006), in Red Palm Weevil *Rhynchophorus ferrugineus* treated with zinc sulfate by Al-Dhafar and Sharaby (2012), in desert locust *Scistocerca gregaria* treated with fenitrothion by Ouali-N'goran (2013) and in *Periplaneta americana* treated with N-nitroso-N-methylurea by Jain & Ahi (2014), whereas 0.005% concentration caused degeneration and distortion in the shape of epithelial and an equivalent result was found in *B. germanica* treated with boric acid by Habes *et al.* (2006), in *Mythimna separata* treated with fraxinellone by Lu *et al*. (2010), in *P. americana* treated with *Datura alba* leaf extract by Khan *et al.* (2011), in the third larval instar of *Chrysomya megacephala* treated with malathion by Bakr *et al*. (2012), in *P. americana* treated with N-nitroso-N-methylurea by Jain and Ahi (2014). At higher concentration the peritrophic membrane was highly degenerated and clustered in the centre of the gut cavity, while at lower concentration peritrophic membrane was less degenerated, not closely lying to the epithelial cells and clustered in the centre of the gut cavity. Similar results were found in *M. separata* treated with fraxinellone by Lu *et al*. (2010), *Spodoptera littoralis* affected by the action of pyridalyl by Dahi *et al*., (2011), in *P. americana* treated with *D. alba* leaf extract by Khan *et al*. (2011), in Red Palm Weevil *R. ferrugineus* treated with zinc sulfate by Al-Dhafar and Sharaby (2012). Outer muscular layer showed no changes indicating no damage in both concentrations of insecticide. In middle and posterior midgut, at higher concentration, the cells were completely ruptured and an equivalent result was found in in larvae of *Culex pipiens* treated with *Eichhornia crassipes* by Assar and El- Sobky (2003) and in the third larval instar of *Chrysomya megacephala* treated with malathion by Bakr *et al.* (2012).

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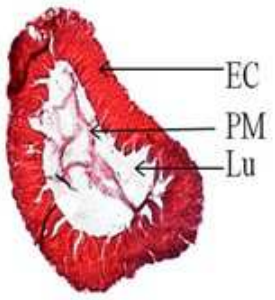

Fig 1: T.S. of midgut of *C.megacephala*: Control (10X)

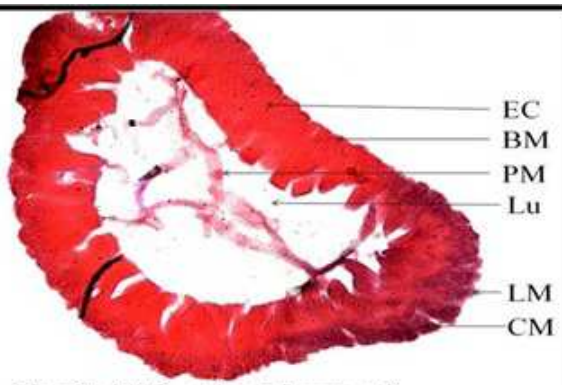

Fig 2: T.S. of midgut of *C.megacephala*: Control (40X)

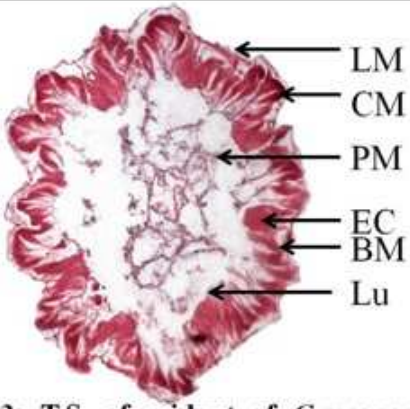

Fig 3: T.S. of midgut of *C.megacephala* treated with 0.005% deltamethrin (10X)

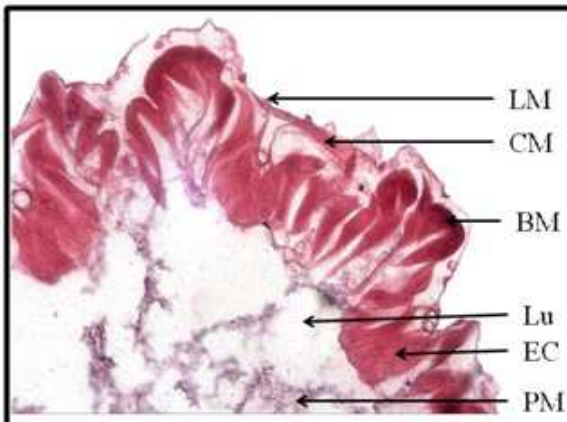

Fig 4: T.S. of midgut of *C.megacephala* treated with 0.005% deltamethrin (40X)

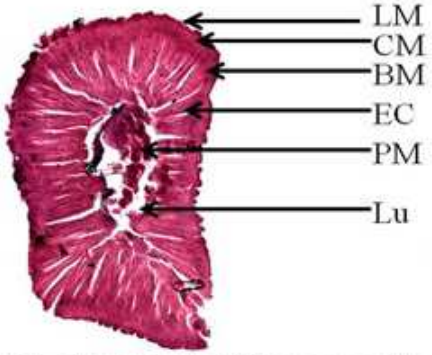

Fig 5: T.S. of midgut of *C.megacephala* treated with 0.05% deltamethrin (10X)

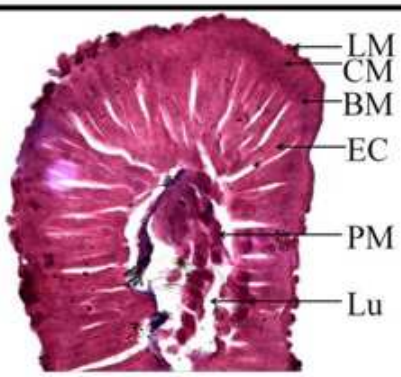

Fig 6: T.S. of midgut of *C.megacephala* treated with 0.05% deltamethrin (40X)

ABBREVIATIONS: BM- Basement membrane; CM- Circular muscles; EC- Epithelial cells; LM- Longitudinal muslces; Lu- Gut lumen; PM- Peritrophic membrane

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