



STUDIES ON HISTOPATHOLOGICAL EFFECT OF CHLOROPYRIFOS ON THE MIDGUT OF AMERICAN COCKROACH, *PERIPLANETA AMERICANA* (LINN.) (DICTYOPTERA: BLATTIDAE)

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ABSTRACT

Periplaneta americana is ancient and highly successful form of free living insect that are found mainly in tropical regions of the world. Cockroaches are considered as medically important insects as they have been shown to harbor pathogenic bacteria, serve as immediate host for pathogenic helminthes, viruses, protozoa and fungi affecting man and various other vertebrates and mechanically transmit disease producing organisms. Chloropyrifos in the concentrations 0.005% and 0.01% was provided in feed of adult cockroaches to observe histopathological changes in midgut after 24 hours. Midgut is lined with enteric epithelium which rest upon a basement membrane and is covered by inner circular and outer longitudinal muscles and contains a thin peritrophic membrane as innermost layer. Histopathological studies on midgut resulted in degradation of basement membrane and distortion in epithelial lining in both concentration (0.01% and 0.005%). Higher dose caused more disruption in longitudinal and circular muscles as compared to low dose. Peritrophic membrane was detached from epithelial lining and almost lost their identity in high dose. Some degree of constriction is observed in lumen in low concentration while at higher concentration shrinkage is more pronounced.

Keywords: Cockroach, *Periplaneta americana*, midgut, histopathology, chloropyrifos.

Introduction

Periplaneta americana is ancient and highly successful form of insect life. The fossil record indicates that they achieved an optimum body from early in evolutionary history and remained as very stable group of insects [Moore *et al.*, 1952]. Cockroaches are free living insect that are found mainly in tropical regions of the world. They live in wide variety of places such as under dead or decaying bark and leaves of trees, under rubbish or stones in caves or burrows, in the nest of ant, wasp, termites or in semi aquatic environment [Roth and Willis, 1960 and Roth, 1973] and feed wide variety of food stuffs including biological waste and garbage [Roth and Willis, 1957; James and Harwood, 1969]. Cockroaches are considered as medically important insects as they have been shown to harbor pathogenic bacteria, serve as immediate host for pathogenic helminthes, viruses, protozoa and fungi affecting man and various other vertebrates [Roth and Willis, 1957, 1960; Tarshis, 1962; James and Harwood, 1969; Rueger and Olson, 1969; Klowden and Greenberg, 1977; Ash and Greenberg, 1980; Cornwell and Mendes, 1981; Fotedar *et al.*, 1991; Cloarec *et al.*, 1992; Kopanic *et al.*, 1994] and mechanically transmit disease producing organisms. *P. americana* excrete mutagenic and carcinogenic compound which are tryptophan derivative [Mullins and Cochran, 1973]. They are hazardous to man as they feed on human food and human feces and can move freely from building to building or from sewer to human habitations [Haines and Palmer, 1955]. Chlorpyrifos is a crystalline organophosphate insecticide, acaricide and miticide. Chlorpyrifos is used around the world to control pest insects in agricultural, residential and commercial settings. Its use in residential applications is restricted in multiple countries. The crops with the most use are cotton, corn, almonds and fruit trees including oranges, bananas and apples. Exposure to various doses of insecticides has affected the alimentary canal in *Schistocerca gregaria* treated with various synthetic insecticides [Mukherji and Haridas, 1954], in *Spodoptera litura* treated with organophosphorus insecticides [Lal *et al.*, 1970], in *Plebiogryllus guttiventris* treated with Fenitrothion [Balakrishnan, 1987], in *Crotogonus trachytrerus* treated with some insecticides [Singh, 1990], in *Oxya nitidula* treated with monocrotophos [Amutha *et al.*, 2005], in *Periplaneta americana* treated with cypermethrin, carbaryl and monocrotophos [Kulkarni *et al.*, 2006], in *Blatella germanica* treated with acetylcholinesterase [Habes *et al.*, 2006], in *Mythima seperata* treated with fraxinellone [Lu *et al.*, 2010], in *P. americana* treated with *Datura alba* [Khan *et al.*, 2011], in *Schistocerca gregaria* treated with fenitrothion [Ouali-N'goran *et al.*, 2013], in *P. americana* treated with N-nitroso-N-methylurea [Jain and Ahi, 2014], *P. americana*

treated with deltamethrin [Majumdar *et al.*, 2016]. Present study investigates histopathological effect of insecticide chloropyrifos on the midgut of cockroach, *P. americana*.

Material and method

Cockroaches were collected during September–December 2015 from sewer manhole in and around the campus of Aligarh Muslim University, Aligarh and cultured at room temperature.

Preparation of insecticidal concentrates:

0.1% stock solution of chloropyrifos was prepared in distilled water. Then this concentration was diluted 10 times to get desired concentration of 0.01%. By mixing 2.5 ml of 0.01% of insecticide solution with 2.5 ml of distilled water 0.005% concentration was prepared.

Application of insecticide:

Bread crumbs were mixed with 3ml of each insecticide concentrations (0.005% and 0.01%) in each petridish which were kept in jars 1 and 2 separately and 5 cockroaches were released in each jar. Parallel to these, control set up was also maintained. Cockroaches were then dissected in Ringer solution's after 24 hours of insecticide treatment to obtain midgut for further studies.

Preservation and histological preparation:

Alimentary canal was excised from junction of foregut and midgut and midgut and hindgut and fixed immediately in Bouin's solution for 24 hours. After 24 hours tissue was washed 2-3 times in tap water to remove excess picric acid and dehydration proceeded in ascending grades of alcohol i.e. 30%, 50%, 70%, 80%, 90% for 5 minutes each while in 96% and 100% alcohol for half an hour each followed by mixture of 100% alcohol and xylene solution (1:1) for 10 minutes. Incubation was done at 60⁰c in xylene and paraffin wax (1:1) for 15 minutes then in pure wax for 2hours.

5 micrometer microtome sections were cut from the prepared block. Then the ribbons were placed on glass slide which was lubricated by albumin solution having 5 drops of glycerin. Slides were then stretched on warming table to remove creases. Slides were processed in 2 changes of xylene and then descending grades of alcohol series 100%, 96%, 90%, 80%, 70%, 50%, 30% for 5 minutes each and then in distilled water for 5 minutes each. Slides were stained in Hematoxylin for 10 seconds, then washed in tap water and counter stained with Eosin for 25

minutes followed by upgrade dehydration in alcohol for 5 minutes each and then 2 changes of xylene for 10 minutes. After air drying slides were mounted using D.P.X to observe under compound microscope. Photographs were taken using LEICA compound microscope using appropriate magnification.

Result and discussion

Normal histology of the midgut of P. americana (Figs. 1 & 2):

Midgut or mesenteron is the longest part of alimentary canal of *P. americana*. Midgut is the main site of secretion, digestion and absorption of the nutrients. Its diameter varies in accordance with the quantity of food it contains. The junction between foregut and midgut, midgut and hindgut is demarcated by origin of hepatic caecae and malpighian tubules respectively. Histologically, structure of midgut shows the following tissues to be much same in size and structure throughout i.e. epithelium of endoderm cells supported by a basement membrane and its apical portion bears striated border, inner circular muscles and outer longitudinal muscles. The cells of epithelium are tall, columnar with distinct cell membranes and conspicuous, centrally placed nuclei. The regenerative cells are small, lie at the bases of functional cells in groups at regular intervals and scattered throughout the length of the ventriculus. Striated circular muscle fibers surround the epithelium. Externally there is a thin connective sheath. The cells of the proximal region are larger, whereas of distal region are flattened.

Effect of 0.005% and 0.01% concentrations of chloropyrifos on midgut of P. americana (Figs. 3-6):

In midgut treated with 0.005% concentration, basement membrane gets degenetated whereas at 0.01% concentration thickening of basement membrane was observed. Similar histopathological changes were observed in *Blatella germanica* treated with acetylcholinesterase [Habes *et al.*, 2006]. Epithelial cells at concentration 0.005% get enlarged and distorted whereas in 0.01% concentration epithelial cells are more effected showing elongation, degeneration, lysis, vacuolization and thickening of individual cells. Similar changes was reported in *P. americana* treated with N-nitroso-N-methylurea [Jain and Ahi; 2014], *Schistocerca gregaria* treated with fenitrothion [Ouali-N'goran, 2013]. Circular and longitudinal muscles cannot be distinguished at 0.005% concentration but at 0.01% concentration distortion is more pronounced. Similar changes

was seen in *P. americana* treated with deltamethrin [Majumdar *et al.*, 2016], in *P. americana* treated with N-nitroso-N-methylurea [Jain and Ahi; 2014], *Schistocerca gregaria* treated with fenitrothion [Ouali-N'goran, 2013]. At 0.005% concentration striated border gets distorted and extended into lumen of midgut whereas in 0.01% concentration striated border get destructed and lost their identity. Similar effects were shown in *Mythima seperata* treated with fraxinellone [Lu *et al.*, 2010]. Peritrophic membrane get constricted and accumulated at center of midgut lumen at 0.005% concentration, but in 0.01% concentration peritrophic membrane disappeared. Similar results were reported in *Mythima seperata* treated with fraxinellone [Lu *et al.*, 2010], in *P. americana* treated with *Datura alba* [Khan *et al.*, 2011].

In conclusion, results obtained from this investigation showed that chloropyrifos caused cellular deformation in the midgut.

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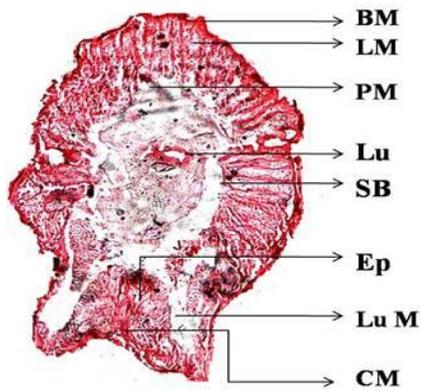


Fig. 1: T.S of junction of midgut and hindgut of *P. americana* : Control (4X)



Fig. 2: T.S of junction of midgut and hindgut of *P. americana* : Control (10X)

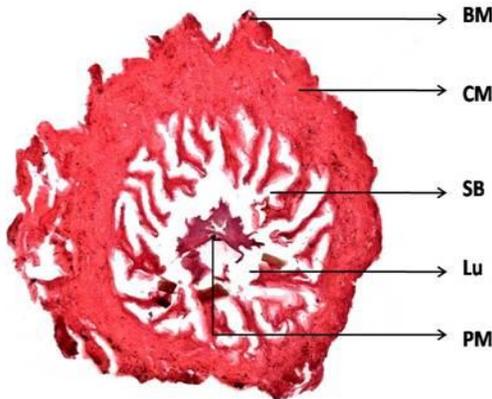


Fig. 3 : T. S. Of midgut of *P. americana* treated with chloropyrifos 0.005% (4X)

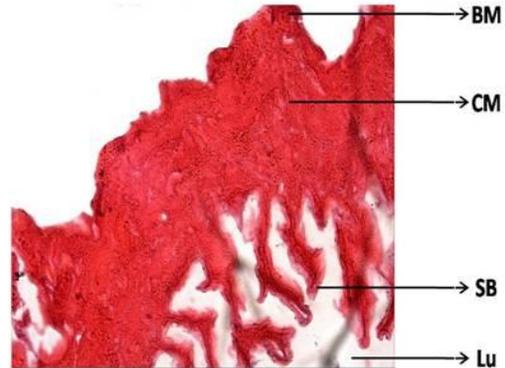


Fig. 4 : T. S. of midgut of *P. americana* treated with 0.005% chloropyrifos (10X)

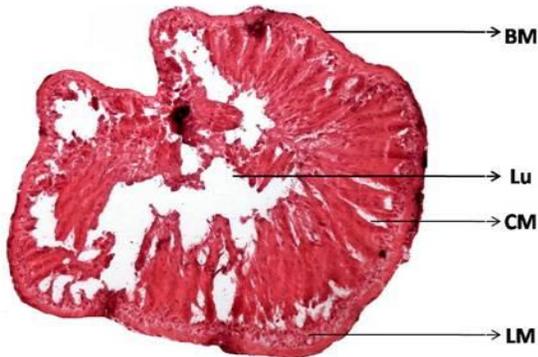


Fig. 5: T. S. of midgut of *P. americana* treated with 0.01% Chloropyrifos (4X)

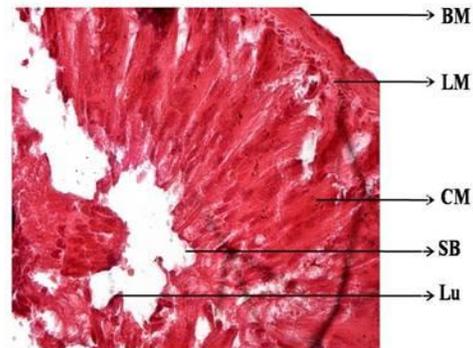


Fig. 6 : T. S. of midgut of *P. americana* treated with 0.01% Chloropyrifos (10X)

Abbreviations: BM- Basement Membrane, CM-Circular Muscle, Ep-Epithelium, LM-Longitudnal Muscle, Lu-Lumen, Lu M-Lumen of Malpighian, PM-Peritrophic Membrane, SB-Striated Border.