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Research Paper

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

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Abstract

Chrysomya megacephala an oriental latrine fly is universally distributed across the extensive area of the Oriental, Australasian, and Oceania regions of the world, and extends as far as Africa, South America and Central America having major role in the forensics, medical and veterinary sciences. Flies are the vectors of various enteric pathogens such as bacteria, virus, protozoa and helminths. They cause various types of myiasis in humans and various mammals. Its eradication is necessary from the vicinity of the humans due to the causative reason of causing the myiasis and various other contagious diseases around the globe. Deltamethrin, in the concentration 0.0005% and 0.005% was provided in the food on the 5th day after the adult emergence to obtain histopathological results on ovaries after 24 hours of treatment. Ovaries consist of terminal filament, germarium and vitellarium. Vitellarium consists of follicles having trophocytes and oocytes enclosed within a capsule formed of cuboidal follicular epithelium having oval nuclei and dense cytoplasm. Treated ovaries shows pycnosis in the cytoplasm, which is more pronounced at higher concentration of insecticide. Vacoulation in the yolk as well as in the trophocytes is evident, with smaller vacoular size at 0.0005% concentration compared to 0.005% concentration. Necrotic trophocytes as well as detachment from the epithelium is distinctly perceptible in the higher concentration. Follicular epithelium is somewhat reduced at lower dose while at the higher dose epithelium is comparably very thin. Yolk is highly deteriorated in case of 0.005% concentration as compared to untreated ovariole. Shape of the ovariole is distorted at higher concentration while in lower concentration, ovariole have somewhat retained the shape.

Key words: *Chrysomya megacephala*, ovariole, histopathology, deltamethrin, pycnosis.

INTRODUCTION

Chrysomya megacephala [Fabricius], an oriental latrine fly [Zumpt, 1965] is considered one of the most dangerous dipteran vector [Wells, 1991]. It is universally distributed across the world having an extensive role in medical, veterinary and forensic sciences

[Smith, 1986; Wells and Kurahashi, 1994; Tantawi *et al*., 1996; De Souza and Linhares, 1997; Centeno *et al*., 2002]. The term "blow" in the name blow fly refers to the habit of females of these flies "blowing" (depositing eggs or larvae on) dead carcasses or live host. Flies are shiny and metallic in colour. Most of them inhabit the carrion, dead organic waste, foul materials, human food [Greenberg, 1971]. Flies are the vectors of various pathogens such as bacteria, virus, protozoa and helminths [Chang, 1943; Pipkin, 1949]. They cause various types of myiasis in humans and various mammals [Zumpt, 1965]. Basic studies on histology of the female reproductive system of *C. megacephala* have been done by Bansal and Murad [1987] and a major research is still to be processed, for its eradication from the vicinity of the humans due to the causative reason of causing the myiasis and various other contagious diseases around the globe. Deltamethrin is a member of synthetic pyrethroids which is safe for mammalian and without known carcinogenic, teratogenic, mutagenic effects. So, the deltamethrin has been employed as an effective insecticide to alter and damage the female reproductive system and thus reduce the fertility. Exposure to the low doses of insecticides has affected and damaged the ovaries in *Poekilocerus pictus* treated with chemosterilants [Saxena and Aditya,1974], in *Sarcophaga ruficornis* treated with thiourea [Chaudhary and Tripathi, 1976], in *Periplanata americana* treated with BHC [Bhide, 1985], in *P. americana* treated with the Y–BHC [Bhide, 1986], in *P. americana* treated with DDT [Bhide,1986], in *P. pictus* treated with endosulfan [Ahi, 1986], in *P. americana* treated with BHC and DDT [Jain and Bhide, 1990], in *P. pictus* treated with BHC [Jain and Bhide, 1991], in *Aedes aegypti* treated with ivermectin [Mahmood *et al.*, 1991], in *P. pictus* treated with endosulfan [Janak, 1992], in *Heteracris littoralis* treated with azadirachtin [Ghazawi *et al.*, 2007], in *Chrotogonus trachypterus* treated with monocrotophos [Shakeet and Bakshi, 2009] and in *C. trachypterus* treated with deltamethrin [Meena and Singh, 2014].

From the literature, it is evident that a very scanty work is done within the dipteran order, so the present study deals with the histopathological effects of the deltamethrin on the ovariole of *C. megacephala.*

MATERIAL AND METHODS

Breeding and Maintenance of *Chrysomya megacephala* –

Chrysomya megacephala were collected from the Aligarh Muslim university campus and brought to the laboratory for research work. The flies were kept in cages made of wire mesh and ply board. Flies were maintained in the BOD cabinet at the temperature 27±2ºC and relative humidity of 60±5%. The adults were reared on the mixture of sugar, protein and milk (1:1:3) soaked in cotton. Chopped buffalo meat as egg laying medium was provided in a petri dish and was replaced daily to maintain hygienic condition. Eggs along with meat were then transferred to the glass jar covered with the muslin cloth and tied with rubber-band to avoid escape of larvae. Larvae were reared on the buffalo meat. Overcrowding was avoided so that they can develop at a normal rate. At 3rd instar larval stage cotton was provided in jar for pupation.

Sampling of Experimental Insects –

On the 5th day of the adult emergence, the flies were treated with the insecticide deltamethrin in the concentration 0.0005% and 0.005% by ingestion method to obtain histopathological results on ovaries of the fly.

Histological Preparation of Ovariole for Light Microscopy –

Control as well treated flies were dissected in insect Ringer's solution under dissecting microscope after 24hrs of treatment. Ovaries were excised and fixed immediately in

bouin's solution for 18hrs and then dehydrated in ascending grades of alcohol 30%, 50%, 70%, 80%, 90%, for 15 minutes each while in 96% and 100% for half an hour each followed by 100% alcohol and xylene solution (1:1) for 10 minutes. Incubation was done at 60°C in xylene and paraffin wax (1:1) for 15 minutes and then in pure wax for 1 hour. An ovariole was then embedded in paraffin wax whose 5 µm microtome sections were cut. Ribbons were placed on glass slide which was lubricated by glycerine and egg albumin solution. The slides were then stretched on warming table to remove creases. Slides were then processed in 2 changes of xylene and then in 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 Delafield's haematoxylin for 10 seconds and then washed in tap water and counterstained with alcoholic eosin for 20 minutes followed by upgrade dehydration of alcohol for 5 minutes each and then 2 changes of xylene for 10 minutes each. Slides were then mounted in DPX to observe under the compound light microscope. Photographs were taken using LEICA compound microscope using appropriate magnification. Histological preparation for the control flies was also done to compare the effects.

RESULTS AND DISCUSSION

Normal Histology of the ovary –

Female Reproductive System of *C. megacephala* consists of paired ovaries, lateral oviducts which joins together to form a common oviduct, 3 spermatotheca and paired accessory glands which opens up separately in the genital chamber. Each ovary consists of 100–110 ovarioles in *C. megacephala* which are polytrophic in nature, enclosed by thin tunica propria in the present study. Similar results were obtained in *C. megacephala* [Bansal and Murad, 1986]. Similarly 75–115 ovarioles were found in *Chrysomya bezziana* [Spradbery and Sands, 1976], near about 100 ovarioles in *Lucilia cuprina* [Clift and McDonald, 1973]. However, Ansari and Murad [1981] found single ovarioles in *Hippobosca maculata.* Ovary of *C. megacephala* consists of outermost follicular layer which is underlined with eggshell, vitelline envelope, trabecular layer, layer of granular material that surrounded the oocyte.

Each ovariole is divided into 3 regions i.e. i) Terminal filament which is short thread like structure, ii) Germarium having primary oogonia which develops into trophocytes and oocytes, iii) Vitellarium consists of many follicles, each having the trophocytes assembled in the upper region and are differentiated into a compact mass of 7 nutritive cells, trophosome and the oocyte [Fig1& Fig2]. Trophosome nourishes the oocyte by rupturing the trophocyte membrane [Gerber *et al*., 1971].

Histopathological effects on the ovaries –

Marked reproductive abnormalities are visible in the treated ovary. Sub-lethal doses of the deltamethrin in the concentration of 0.0005% [Fig 3 & Fig 4] and 0.005% [Fig 5 & Fig 6] caused damages and distortion at various places within the ovariole. Yolk bodies shows deterioration and degeneration of oocytes which are highly pronounced at higher concentration and a comparable results is obtained in *Dysdercus cingulatus* treated with tepa [Sukumar and Naidu, 1973], in *Locusta migratoria* treated with tepa [Nath *et al*., 1975], in *Periplanata americana* treated with BHC and DDT [Jain and Bhide, 1990], in *Philosamia ricini* treated with thiotepa [Mohapatra, 2007], In *Poekilocerus pictus* treated with fenoxycarb [Rai *et al*., 2011]. At the higher concentration the shape of the ovariole is immensely altered while at the lower concentration the ovariole has somewhat retained the shape and an identical results was obtained in *P. ricini* treated

with thiotepa by Mohapatra [2007]. The wall of the ovariole shows rupturing and degeneration at various places. At the lower dose [0.0005%], ovariole sheath as well as follicular epithelium is reduced while at the higher dose [0.005%], the follicular epithelium shows thinning of the membrane and thus detaches from the trophocytes. An equivalent results were observed in *Chrotogonous trachypterus* treated with monocrotophos [Shakeet and Bakshi, 2009], in *Sarcophaga ruficornis* treated with cypermethrin [Amir, 2014]. The germarium shows obliterated and necrotic trophocytes, vacuoles are present in between the trophocytes as well within the yolk at the lower dose, while at the higher dose the vacoular size is very large occupying most of the space. Similar results has been shown in *P. pictus* treated with chemosterilants [Saxena and Aditya, 1974], in *P. americana* treated with BHC [Bhide, 1986], in *P. pictus* treated with the endosulfan [Janak, 1992], in *P. ricini* treated with thiotepa by [Mohapatra, 2007] and also in *Heteracris littoralis* treated with the azadirachtin [Ghazawi *et al*., 2007].

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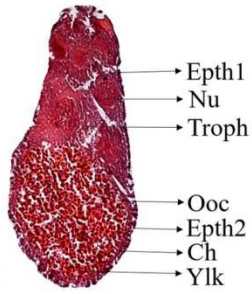

Fig 1 : L.S. of a control ovariole (10x)

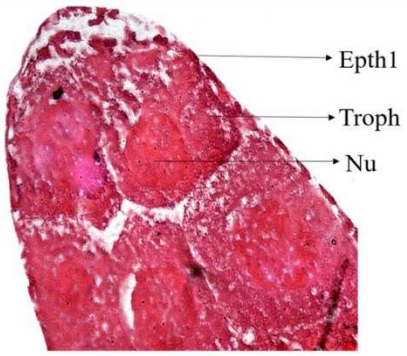

Fig 2 : L.S. of a control ovariole (40x)

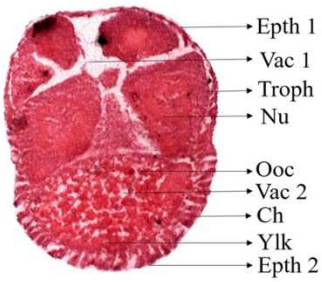

Fig 3 : L.S. of an ovariole treated with 0.0005% Deltamethrin (10X)

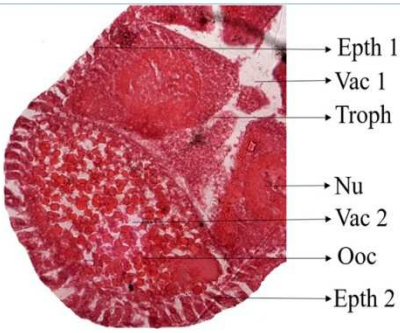

Fig 4 : L.S. of an ovariole treated with 0.0005% Deltamethrin (40X)

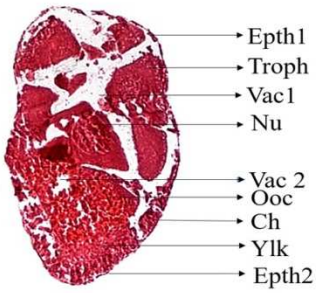

Fig 5 : L.S. of an ovariole treated with 0.005% Deltamethrin (10x)

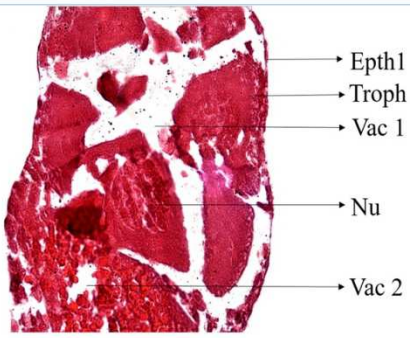

Fig 6 : L.S. of an ovariole treated with 0.005% Deltamethrin (40x)

ABBREVIATIONS : Ch – Chorion, Epth 1 – Follicular epithelium around trophocytes, Epth 2 – Follicular epithelium around oocyte, Nu – Nucleus, Ooc – Oocytes, Troph – Trophocytes, Vac 1 – Vacuole in trophocytes , Vac 2 – Vacuole in yolk, Ylk – Yolk.