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Histochemical study of *Pila globosa* gill when exposed to sub-lethal dose of Monodhan 36% S. L. (Monocrotophos) and Radar 20 EC (Chlorpyrifos)

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ABSTRACT

In the present investigation histochemical study of *Pila globosa* gill was taken up in order to assess the extent of damage of biomolecules i.e glycogen and protein at tissue level. Glycogen level was considered because it is the storage carbohydrate and important source of energy during hibernation of snails and protein being found in large amount in *Pila globosa*. Gill was examined because it has high sensitive to tissue acetylcholinesterase. In the present study, it was observed that Radar 20 EC has more deleterious effect on gill in comparison to Monodhan 36% S. L. Both glycogen and protein level declined. The present work highlights that pesticides exposed *Pila globosa* is not good for human consumption or as bait.

INTRODUCTION

Pila globosa belong to class gastropoda of phylum mollusca. They are soft bodied animals enclosed in calcareous shell. Being enclosed inside hard, less permeable shell, they are considered to be hardy animals that can tolerate high level of toxicity but actually it is far away from fact (Benedetti *et al*., 1982, Ahirrao and Kulkarni, 2011). In the present investigation, assessment of the toxicity induced by commercial grade chlorpyrifos by the brand name of Radar 20 EC and monocrotophos by the brand name of Monodhan 36% S. L. on *Pila globosa* was studied especially on histochemical parameters of glycogen and total protein. These pesticides are widely used for agricultural purposes and for treating soil against different variants of soil borne insects, mites and on foliar application. Chlorpyrifos and monocrotophos are contact (non-systemic) organophosphate insecticides. They irreversibly bind with acetylcholinesterase enzyme thereby inhibiting signal transmission along neurons. They affect neurons and muscular junction of the organisms (Sánchez-Bayo, 2012). They are broad spectrum insecticide and highly poisonous to bees, mammals and birds and aquatic organisms (Rehman *et al*., 2012, Jameson *et al*., 2006, Moore *et al*., 2014, Giesy *et al*., 1999 and Swamy *et al*., 1992). Gill is highly sensitive to tissue acetylcholinesterase (Moser, 2011 and Rao *et al*., 2005), hence selected for present investigation.

MATERIALS AND METHODS

Model Animal:

Pila globosa (Swainson) is a common snail found in and around Dumka. This animal is active in rainy season and is easily procurable from April to September, but after September it undergoes hibernation. However, on the moist banks of ponds, the animal is easily available up to the first week of November, if temperature is above 16°C. *Pila globosa* were collected from ponds meant for personal use. They were brought in the laboratory and acclimatized for the next 72 hours.

Pesticides Used:

Monodhan 36% S. L. and Radar 20 EC belonging to monocrotophos and chlorpyrifos group of pesticides were used. Both the pesticides were procured from local market.

Experimental Design:

Acclimatized mature snails of average weight 12-15 grams and length 5-7 centimeters were selected and put in plastic tubs in 5 liters of water. Plastic tubs were covered with mosquito net to avoid escaping of tested animals out of tub. Four tubs were taken with 10 snails in each tub. Two tubs were considered as control and other tubs were used for pesticide assessment. In one tub $1/10^{th}$ concentration of 72 hours LC_{50} value of Monodhan 36% S. L. (monocrotophos) was added and in other tub $1/5^{th}$ concentration of 72 hours LC_{50} value of Radar 20 EC (chlorpyrifos) was added. The added amount of Monodhan 36% S. L. and Radar 20 EC was 48.3 ppm and 17.8 ppm, respectively. Toxicity of chlorpyrifos was more in comparison to monocrotophos hence more diluted amount of chlorpyrifos was added. Animals were given selected doses for 15 days. Every day, water of each tub was changed and fresh toxicants were mixed in each tub except control ones. On the 16^{th} day animals were put out of water. Gills of control snails were dissected out along with Monodhan 36% S. L. (monocrotophos) and Radar 20 EC (chlorpyrifos) treated snails for histochemical study. The experiment was replicated thrice. For histochemical demonstration of glycogen and protein Best's Carmine test (McManus and Mowry, 1958) for glycogen and Mercuric bromophenol method (Mazia *et al*., 1953) for proteins test was performed after preparation of slides.

RESULTS AND DISCUSSION

Histochemical observation of glycogen in gill of *Pila globosa* showed Intra-lamellar spaces (ILS), Frontal cells (FC), latero- ventral cells (LVC), absorptive cells (AC), Endothelial cells (EC) and cilia (C) in control. In Monodhan 36% S. L. (monocrotophos) treated gill of *Pila globosa* Frontal cells with cilia are faintly visible and stained blue while Endothelial cells (EC) are found to be slightly damaged and stained purple. In Radar 20 EC (chlorpyrifos) treated gill of *P. globosa*, degeneration of cilia (C) is clearly evident from frontal cells (FC). Swelling at the tip of gill lamellae can be observed. One ruptured tip due to swelling is evident. Degeneration of frontal cells (FC) is more in comparison to latero-ventral cells (LVC).

Histochemical observation of protein in gill of *Pila globosa* showed Intra-lamellar spaces (ILS), cilia (C) in addition to protein rich granules (PRG) in control slides. In Monodhan 36% S. L. (monocrotophos) treated snail's gill lamella is found to be damaged. Slight degeneration of cilia from few frontal cells is noticeable. Endothelial cells are not visible. Protein rich granules (PRG) are light stained in comparison to control. In Radar 20 EC (chlorpyrifos) minor swelling of lamellar tip can be noticed. Degeneration of few cilia is clearly evident from frontal cells (FC). Protein rich granules (PRG) are stained sky blue. Cilia (C) is found to be present only in latero-ventral cells (LVC).

In Carmine stained Monodhan 36% S. L. (monocrotophos) treated *Pila* globosa permanent slides, degeneration of gill started as absorptive cells began rupturing. Skeletal rod was found to leave the inner wall of lamella. Endothelial cells appeared slightly damaged. In Radar 20 EC (chlorpyrifos) treated *Pila globosa*, swelling of the lamellar tip and its rupture was obvious. Degeneration of gill filament cilia was observed along with the degradation of the frontal cells. Ruptured cells were accumulated in inter-lamellar space. Degree of gill damage is more severe in Radar 20 EC (chlorpyrifos) when compared with Monodhan 36% S. L. (monocrotophos) treated gill. In Mercuric Bromophenol blue stained Monodhan 36% S. L. (monocrotophos) treated *Pila globosa*, slight deformities were noticed in lamellar cells and endothelial cells of gill filament. In Radar 20 EC (chlorpyrifos) treated *Pila globosa*, slight swelling in the tip of lamella was noticed. Degeneration of frontal cells, latero-ventral cells

and absorptive cells along with complete loss of cilia was evident. Radar 20 EC (chlorpyrifos) showed higher level of degeneration in gill when compared with Monodhan 36% S. L. (monocrotophos) treated snail gill.

Photomicrographs of carmine stained gill of *Pila globosa* (400X)

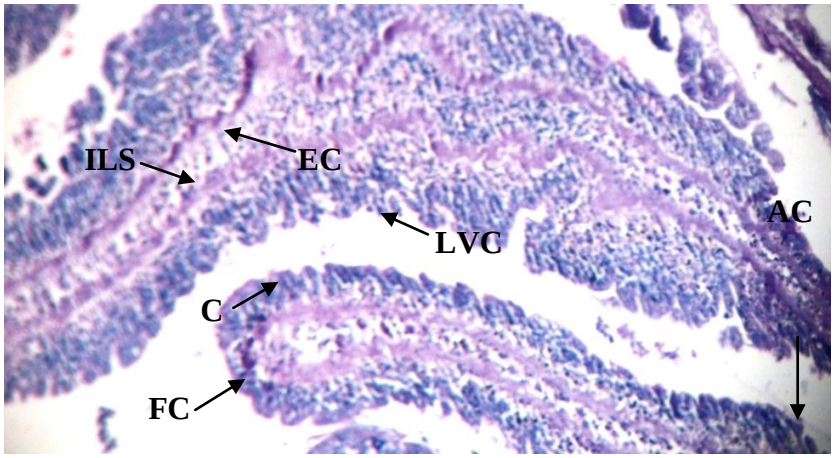

Fig. 1: Control gill stained with carmine. EC (endothelial cell), AC (absorptive cell) LVC (latero-ventral cell), C (cilia), FC (frontal cell), ILS (intra-lamellar space).

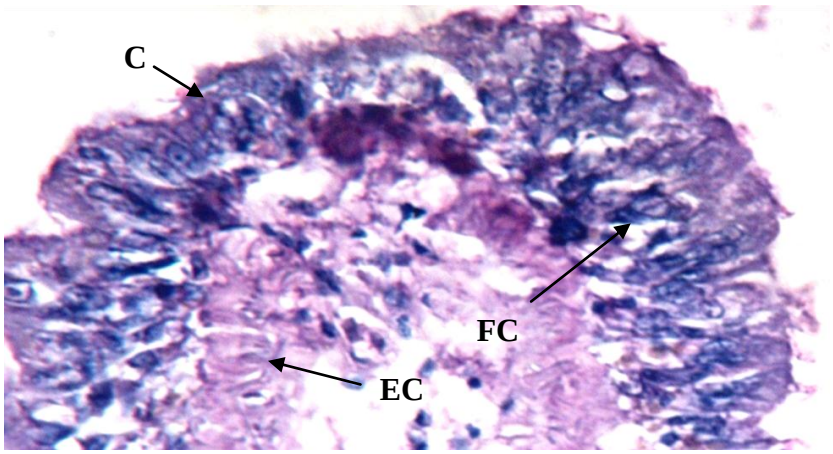

Fig. 2: Monodhan 36% S. L. treated gill stained with carmine. EC (endothelial cell), C (cilia), FC (frontal cell). FC and EC are shown to be disrupted.

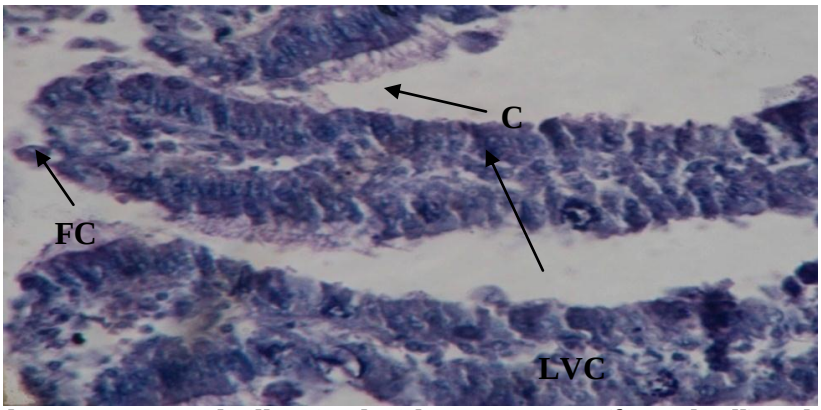

Fig. 3: Radar 20 EC treated gill stained with carmine. FC (frontal cell) is shown to be degenerated, LVC (latero- ventral cells) is malformed but intact and C (cilia) is absent from most of latero-ventral cells (LVC).

Photomicrographs of Bromophenol blue stained gill of *Pila globosa* (400 X)

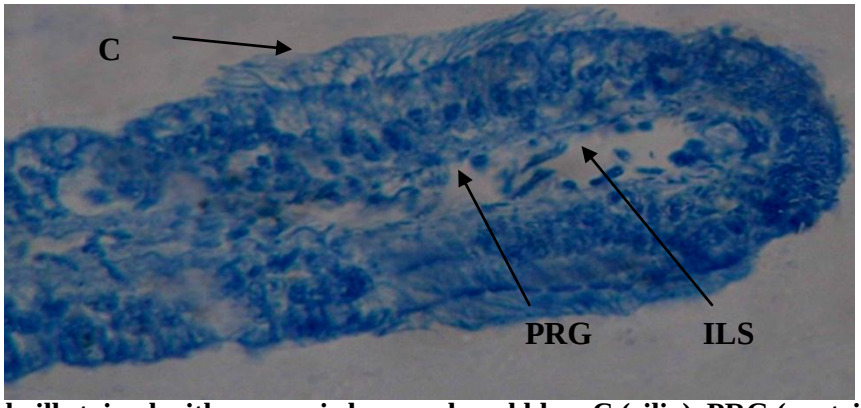

Fig. 4: Control gill stained with mercuric bromophenol blue. C (cilia), PRG (protein rich granules), ILS (intra-lamellar space).

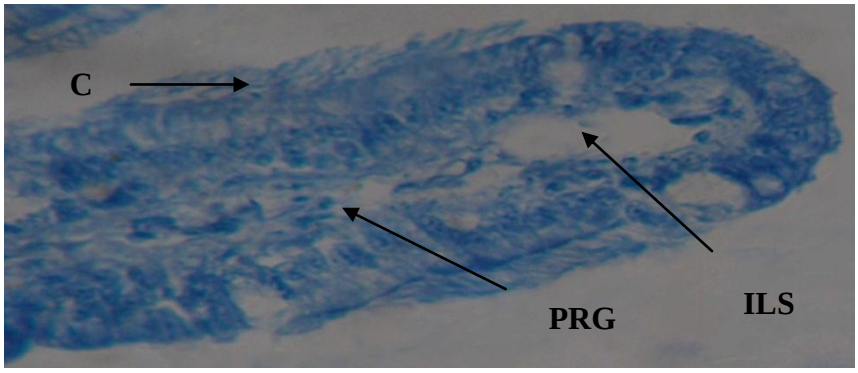

Fig. 5: Monodhan 36% S. L. treated gill stained with mercuric bromophenol blue. PRG (protein rich granules reduces drastically while ILS (intra-lamellar space increases.

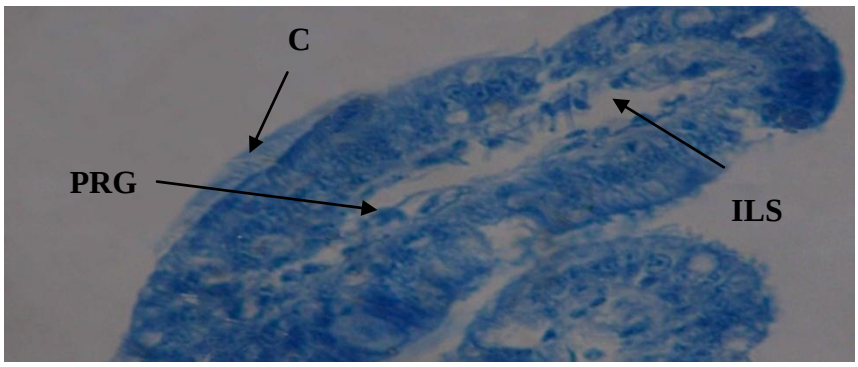

Fig. 6: Radar 20 EC treated gill stained with mercuric bromophenol blue. C (cilia started clumping. PRG (protein rich granules) are found to be clumped. Slightly swollen tip of gill lamella is visible.

Pila is an amphibious mollusca and therefore, it is particular in having both aquatic and aerial modes of respirations. Generally, the snail respires alternatively with the aquatic and aerial modes of respiration but one mode may be preferred to the other according to the conditions. In the present study, test animals were kept in water filled tubs, so aquatic mode of respiration was preferred. Ctenidium or gill is responsible for aquatic respiration. In the histochemical study of the gill of control snail, endothelial cells were strongly carmine positive along with cilia. Frontal cells, latero-ventral cells and absorptive cells showed less

affinity with carmine. Same type of reactivity was noticed in monocrotophos treated gill of mud snail though histological distortion was evident. No characteristic colour of carmine was observed in chlorpyrifos treated gill section. Distorted cells were blue in colour. Epithelial cells of gill of control animal were highly positive for mercuric bromophenol blue while endothelial cells showed negative results and did not stain at all. Epithelial cells of monocrotophos and chlorpyrifos treated snail's gill showed weak reactivity towards mercuric bromophenol blue. Distortion of cells was clearer in chlorpyrifos treated gill's section with lamellar apex swelling.

Chlorpyrifos is a long acting inhibitor of acetylcholinesterase as described by Zou *et al*. (2014) on *Pomacea canaliculata*. A high acetylcholinesterase activity in the gill was observed by Mora *et al*. (1999) in *Mytilus galloprovincialis*. Furthermore, they suggested the presence of gill ganglion that innervates the gill for high acetylcholinesterase activity. Putkome *et al*. (2008) reported the highest acetylcholinesterase activity in gill followed by intestine, muscle, kidney and digestive gland, respectively in *Pomacea canaliculata* exposed to chlorpyrifos, dichlorvos or carbaryl insecticides. This effect is confirmed by the histochemical study of gill that led to the tissue degeneration. Gill of *Pila globosa* is affected by chlorpyrifos with greater severity in comparison to monocrotophos. Histochemical studies revealed the decrease in glycogen and protein in the tissues with increased degeneration in treated snails in comparison to untreated ones.

Hence, it is advisable to avoid pesticides exposed apple snails' use as bait or for human consumption. *Pila globosa* from pesticide sprayed fields should not be picked up for eating.

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