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The Effect of Paraquat (Herbicide) on the Mantle of Freshwater Snail *Lymnaea luteola* (Lamarck: 1799)

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Abstract

Mantle cavity act as a respiratory organ in most molluscs and is the specialized organ of the gastropods for it not only bears the glands that secrete the shell but also acts as buffer between the hard calcareous shell and the animal itself, spreading like a cushion through the entire inner surface of the body whorl. It is highly muscular, thin and bound by a single layer of epithelial cells. The present study was designed to investigate the histopathological effect of paraquat on the mantle of freshwater snail *L.luteola*. Acute toxicity of concentration of paraquat to *L.luteola* was determined by recording the snail mortality of 24,28,72 and 96 hrs exposures. The LC_{50} value based on probit analysis was found to be 0.073 ml/L for 96 hrs of exposure to the herbicide. Microscopical examination of treated animals revealed severe structural alterations in the mantle of *L.luteola*. The glandular cells exhibited a mild to heavy bioaccumulation of the herbicide which was a time dependent effect. At various concentrations paraquat causes significant dose dependent changes such as : desquamation of epithelial cell, atrophy of muscle fibers, necrosis, in respect of glandular cells.

Keywords: Paraquat, *Lymnaea luteola*, Mantle, Histopathology, Toxicity

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1. Introduction

In recent years aquatic ecosystems are exposed to pesticides and herbicides that enter the water way from various sources and contaminate different compartments of the ecosystem [1-5]. Because of their aquatic distribution, pesticides affect a wide range of non target organisms, like invertebrates and fishes, those inhabiting aquatic environment [6, 7]. The rate of increase of pesticide and herbicide use is relatively high in India. For centuries, agro chemicals, especially herbicides are routinely employed as part of the integrated farming practice to protect crops from insects, weeds and diseases. Paraquat dichloride (1, 1'- dimethyl -4, 4'-bipyridinum dichloride) is a one of the widely used non selective contact herbicide in the state of Andhra Pradesh (East Coast of India, 17° 36' N; 83° 0' 0E.) for aquatic weed control in Rice. It is extremely soluble in water and contains various wetting agents (condensation products of ethylene oxide and alkyl phenols), spreaders and plant adhesion materials (Carboxyl methylcellulose, Polymethacrylates). It has been reported that at high concentrations paraquat cause embryo toxic alterations in the *Xenopus laevis* [8-9].

Lymnaea luteola is a freshwater snail, belongs to Lymnaiedae family, and is distributed all over the world including India and it is rarely found in Andhra Pradesh. It is one of the most common species in Kondakarla Lake of Visakhapatnam. Agricultural activities are more common in this region. Around the lake there is extensive cultivation of rice and farmers extensively use Paraquat to control aquatic weeds. The present study deals with the toxicity of paraquat on the snail *Lymnaea luteola* especially histopathological alterations. Because they are common and non – target organisms in the environment, it has been considered a suitable experimental model. Some data are available on the effects of chemicals such as copper sulphate [10, 11] mercury, copper [12] Phenols [13] zinc [14] and plant molluscicides on the fresh water snail *Lymnaea luteola*. There is no information on histopathological effects in *L.luteola*, exposed to herbicides. This species

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gives more sensitive response to chemicals than those of some other aquatic snails since they are non- operculates. Therefore, in a laboratory investigation, we have studied the histopathological effect of exposure to various doses of non-selective herbicide (Paraquat) on the mantle of *L. luteola*. The histopathological alterations in tested snails are made known, in order to determine the potential use of paraquat in the freshwaters surrounded by agricultural fields.

2. Materials and Methods

2.1 Field sampling and Maintenance of the stock specimens

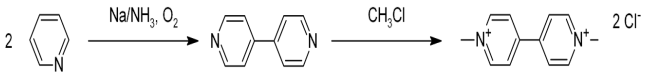
The snails *Lymnaea luteola* were collected from Kondakarla Lake near to the town of Visakhapatnam, Andhra Pradesh, East coast of India. They were collected by small nets and directly by hand picking from natural substrata and stored in a 25-l plastic tank filled with lake water, and immediately transported to the laboratory. Once in the laboratory, animals were cleaned and maintained in glass aquaria at room temperature and exposed to natural photoperiod (12hrs light and 12hrs dark). Specimens were acclimated to laboratory condition for 2 days before the experiments. They were kept in continuously aerated water with a static system. The water in the aquaria was renewed once a day to provide freshwater rich in Oxygen.

2.2 Chemicals used

For the assays, a commercial formulation of the herbicide Paraquat(PQ) with the trade name Gramoxene employed in agriculture applications was used. It most commonly used as non selective contact herbicide in and around the Visakhapatnam district, East Coast of India

2.3 Production

Pyridine coupled with sodium in anhydrous ammonia to give 4,4' – bipyridine , which is the methylated with chloromethane to give the desired compound.



2.4 Experimental design

For experimental purposes, active, healthy, snails of equal size groups were selected. Depending on the purity of the herbicide, required concentration was made by dissolving it in distilled water. Test solution is prepared by dissolving 1ml of herbicide (Paraquat) in one Liter of water (1% solution) and it was taken as stock solution. Required concentrations of the test solutions were prepared by dilution. Only actively moving animals of similar size were selected from the collection for testing.

Preliminary screening tests were conducted to obtain the range of lethal concentrations and based on these, the experiments were carried out. For each concentration ten acclimated snails were used in each aquarium containing different concentrations of herbicide as well as in the control as described by Rahman respectively [15]. Controls are integral part of any toxicity test and necessary to determine if organism's death is due to the toxicant or some other factor. The experimental and control media were renewed daily with freshly prepared solution and water was aerated throughout the experiment. Each exposure was repeated 3 times to obtain consistent results with the same toxicant concentration. The experiment was carried out in the laboratory at room temperature of 28.1°c., acute toxicity tests were performed under static conditions up to 96 hours by recommended methods. Controls were maintained for the same period as the experimental animals. Death was defined as a complete immobilization and failure of the animal to respond to prodding. Dead animals were removed daily from each concentration. No mortality was observed in the control group.

The toxicants were expressed as Lc_{50}. i.e. the calculated concentration of a chemical in water that produced 50% mortality to test organisms during a specified time. The median lethal concentration (Lc_{50}) was computed using probit analysis [16]. In this analysis the percentage of mortality rates were converted into regression lines were drawn between log concentration and probit values.

2.5 Preparation of Tissue samples for Histopathological study

At the end of each exposure period, dead animals were promptly removed from each exposure concentration and control. After the removal of the shells, tissues were fixed in Susa. The tissues were processed according to routine histological methods dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 7-8 µm thickness by rotary microtome. The sections were stained with different histological techniques for observation of histopathological changes in mantle of

snails by using light microscope and digital images were captured with a Sony digital camera. The size (diameter) of mucocytes and protein cells were taken for the control and exposed, groups from the slides using ocular micrometer. The size of the cells data is presented as the mean ± standard deviation. The use of the histological terminology was followed by Hyman [17].

3. Results

Acute toxicity of series of concentration of paraquat to *Lymnaea luteola* was determined by recording snail mortality of 24, 48, 72 and 96 hrs exposures. The Lc_{50} values along with standard error, 95% fiducial limits of the snail, *Lymnaea luteola* for 24, 48, 72 and 96 hours have been calculated and shown in table – 1.

The Lc_{50} value based on probit analysis was found to be 0.073 ml/L for 96 hrs of exposure to the herbicide. Results obtained shown that there were no mortality of snail either in control and those exposed to 0.0196 ml/L paraquat throughout the 96 hrs. Whereas 100% mortality was recorded with 48hrs, on exposed to 0.790ppm concentration of stock solution of paraquat. The results show that Lc_{50} for 24hrs is 1.20 ppm and for 48 hrs is 0.361 ppm while the safe concentration for 72 hrs is 0.220 ppm and for 96 hrs 0.073 ppm.

3.1 Toxicity symptoms:

The behavior of the control snails was normal. Immediately, the herbicide was introduced into the test animals in different aquaria at different concentration level, abnormal behavior such as erratic swimming, restlessness, excessive secretion of mucous, rolling movement (at high concentrations) were observed. Most of the snails tended to move to the upper side of the leaves or glass wall of container above the level of water. After some time, they become weak, lethargic and lose equilibrium completely as a result of which they sink to the bottom of the glass container lying on their sides for considerable longer time. There was discoloration on the body shell of the snails at high concentrations. Normal color and behavioral responses were observed in the control animal, the color became pale at high concentrations at the end of 96 h of exposure time to the herbicide.

3.2 Histopathlogical changes in the mantle

The results show the histological alterations of the mantle of *L. luteola* treated with paraquat. The tissue is slightly damaged after exposure to acute toxicity (96hrs) of paraquat. The outer margin of the control animal group shows that the mantle of *L.luteola* has a few scattered skeletal muscle fibers lying between a columnar epithelium on the distal surface (against the shell) and simple cuboidal epithelium layer lining the mantle cavity. Beneath the epithelium of the inner fold is a layer of glandular area, which contains two types of cells, one of them faintly, stained while the other is darkly stained with eccentric nuclei and has secretory nature (Fig.1). Paraquat cause significant histopathological changes in the mantle of *L.luteola*. The glandular cells exhibited a mild to heavy bioaccumulation of the herbicide which was a time

Table 1. Comparison of lethal concentrations of Paraquat for *Lymnaea luteola*. Each value represents the concentration ± standard error. The values in the parentheses represent 95% fiducial limits.

Lethal concentrations	Concentration (ppm) ± SE			
	24 hrs	48 hrs	72 hrs	96 hrs
Lc_{5}	0.5208 ± 0.062 (0.458 – 0.583)	0.1312 ± 0.034 (0.097 – 0.0178)	0.081 ± 0.014 (0.067- 0.095)	0.020 ± 0.004 (0.016 - 0.024)
Lc_{10}	0.6265 ± 0.071 (0.555 – 0.697)	0.1640 ± 0.014 (0.150 – 0.178)	0.101 ± 0.047 (0.055 - 0.147)	0.026 ± 0.004 (0.022-0.030)
Lc_{25}	0.8522 ± 0.072 (0.7802 – 0.9242)	0.2387 ± 0.063 (0.175 – 0.270)	0.145 ± 0.016 (0.129 - 0.161)	0.043 ± 0.005 (0.038 - 0.048)
Lc_{50}	1.20 ± 0.083 (1.117 – 1.283)	0.361 ± 0.096 (0. 265 – 0.458)	0.220 ± 0.018 (0.202 - 0.237)	0.073 ± 0.007 (0.066 - 0.080)
Lc_{75}	1.68 ± 0.134 (1.546 – 1.814)	0.5490 ± 0.046 (0.502 – 0.596)	0.330 ± 0.028 (0.302 - 0.358)	0.125 ± 0.013 (0.112 - 0.138)
Lc_{90}	2.29 ± 0.234 (2.56 – 2.524)	0.798 ± 0.068 (0.730 – 0.870)	0.482 ± 0.067 (0.414 - 0.549)	0.203 ± 0.028 (0.175 - 0.231)

dependent effect. A mild bioaccumulation of paraquat was detected after 24 hrs in the glandular cells. After 46,72hrs and 96 hrs of exposures the animal's tissue became more affected (Fig.2) and glandular cells appear dark, indicating heavy bioaccumulation of herbicides. Increased amounts caused desquamation of epithelial cells, atrophy of muscle fibers, necrosis, in respect of glandular cells (Fig.3&4). Epithelial cells showed decreased staining ability. The tissue and cell types investigated reflect potential sensitive indicators of an adaptive response in *Lymnaea*. After exposure of paraquat the glandular cells of the mantle changed gradually from mild to heavy dark appearance.

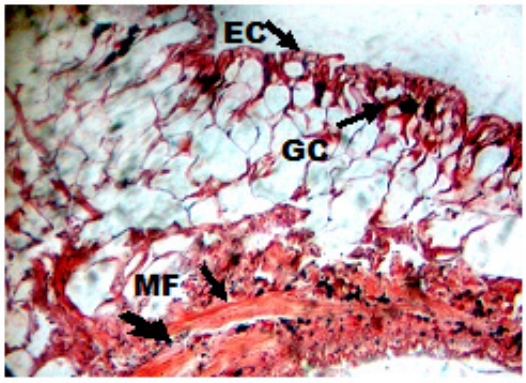


Figure 1. Sagittal section of mantle (control) of *Lymnaea luteola* showing arrangement and shape of muscle fibers, epithelial cells and gland cells. (Azan)
GC - Gland Cells
EC - Epithelial Cells
MF - Muscle Fibers

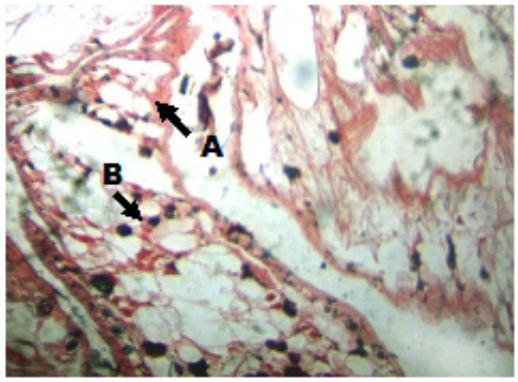


Figure 2. Sections showing mantle of *Lymnaea luteola* exposed to 0.07311 ml/ L Paraquat for 96 hrs (Azan)
A. Slightly ruptured epithelial cells with granulated cytoplasm
B. Glandular cells are dark

4. Discussion

The study of the fresh water snail *Lymnaea luteola* exposed to pollutant indicated that all the chemicals did not exhibit the same effect. Different concentrations caused variation in survival rates because; several factors depend on the toxicity and susceptibility of the animal. The toxic effects are first seen by change in the behavior of which are followed in more cases by death. The exposure of aquatic organisms to even very low levels of pesticides in their environment may result in various biochemicals, physiological and histological alterations in vital tissues of aquatic organisms [18, 19]. As seen in histological

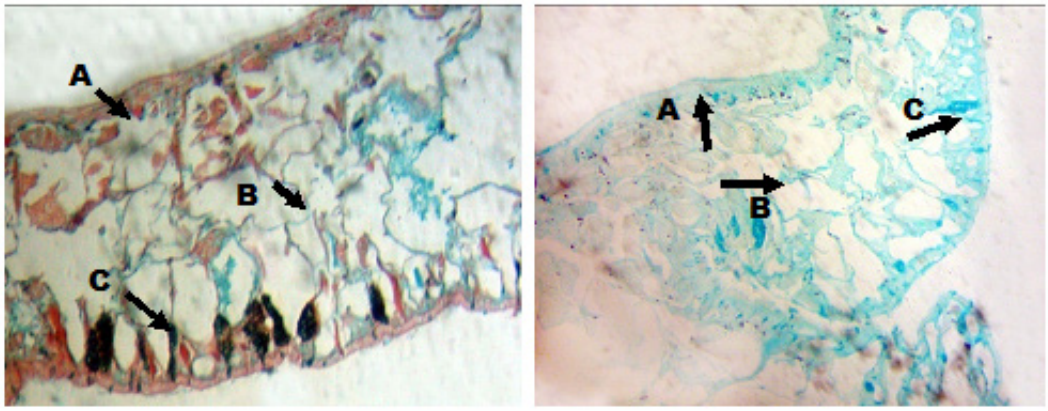


Figure 3 & 4. Sagittal section of mantle exposed to 0.07311 ml/ L paraquat for 96 hrs (AB/PAS and AB)
A. Desquamation of epithelial cells
B. Atrophy of muscle fibers
C. Degenerated and necrotic glandular cells

preparations of snail *Lymnaea luteola*, the control histological structure of the mantle showed an agreement with the pulmonate basic structure given by Hyman [17]. Generally, no histopathological alterations were found in control snails. However, serious histopathological changes were found after exposed to paraquat when compared with control. There is little information on the histopathological effects of herbicide on the mantle of freshwater snail *Lymnaea luteola.* The mantle is an important organ for respiration in pulmonata [20]. A special adaptation of the mantle allows them to take oxygen in air and this is undoubtedly advantageous when the available oxygen in the water is low. The histopathological changes seen in the present study confirmed that paraquat affected mantle mucocyte cells and protein cells. The mantle of *L.luteola* exposed to 2.29 to 0.073 ppm concentration for 96 showed, that the number of mucocytes and protein cells were changed when compared to those in the control snails. On the other hand, decrease in the number and size of mucocytes and protein cells were observed in the 0.073 ml/L paraquat for 96 hrs compared to control snails. Increased mucous production followed by increased mucous secretion is one of the first reactions of gastropods to many kinds of stressors, including mechanical stimuli or chemical irritation caused by mollucicidal chemicals [20]. Similar histopathological changes were also found in the mantle of the freshwater snail *Viviparus bengalensis* [21] showed disorganization of the mantle tissues, especially in the lamellae of *Bellamiya dissimilis* exposed to endosulfan, methyl parathion, quinalphos and nuvan. Otludil et al [3] also have been reported histopathological changes in the mantle tissues of great ramshorn snail *Planorbarius corneus*. The observations of Erhan. Unlu et al [22] gave information on the histopathological effects of mantle exposed to Thiodon in *Lymnaea stagnalis*. Similarly Cengiz et al [23, 24] observed histopathological effects of mantle in *Galba truncatula*, exposed to the pesticide Thiodan. Recently Nahla. EL – Shenawy et al [5] give detailed account on histopathological alterations of mantle in Clam, *Ruditapes decussates* by exposed to organophosphorous pesticides Reldan and herbicide Roundup.

5. Conclusion

The mantle is an important respiratory organ in molluscs. Paraquat is a common herbicide used by farmers to aquatic weed. It caused significant histopathological alterations in the mantle of the snail based on its exposure period. The toxic effects are first seen by change in the behavior of which are followed in more cases by death. The histopathological examinations reveal the desquamation of epithelial cells, atrophy of muscular fibers and necrosis of glandular cells. All the histopathological observations indicated that exposure to sub lethal concentrations of paraquat caused destruction of tissue of *L.luteola.*

6. Conflict of Interest

None Declared

7. References

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