Histopathological Effect of Paraquat (Gramoxone) on the Digestive Gland of Freshwater Snail *Lymnaea luteola* (Lamarck: 1799) (Mollusca: Gastropoda)

Vijaya Kumar Kanapala¹*, Sowjanya Priyadarsini Arasada²

¹Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India - 530003
²Department of Zoology, St. Joseph’s College for Women (A), Gnanapuram, Visakhapatnam, Andhra Pradesh, India - 530004

*Corresponding Author: vijaydarsin@yahoo.com, vijayzool143@gmail.com

Received 4 July 2013; Accepted 10 August 2013

Abstract. Histopathological alterations induced by paraquat in the digestive gland of the freshwater snail *Lymnaea luteola* were investigated. Samples were collected from the Kondakarla lake (Visakhapatnam, Andhra Pradesh, India), where agricultural activities are widespread. 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 LC₅₀ value based on probit analysis was found to be 0.073 ml/L for 96 hrs of exposure to the herbicide. Results obtained showed that there were no mortality of snail either in control and those exposed to 0.0196 ml/L paraquat throughout the 96 hrs. 100% mortality was recorded with 48hrs on exposed to 0.790 ppm concentration of stock solution of paraquat. At various concentrations paraquat causes significant dose dependent histopathological changes in the digestive gland of *L. luteola*. The histopathological examinations revealed the following changes: amebocytes infiltrations, the lumen of digestive gland tubule was shrunken; degeneration of cells, secretory cells became irregular, necrosis of cells and atrophy in the connective tissue of digestive gland.

Key words: *Lymnaea luteola*, Herbicide, Mollusca, Digestive gland, Toxicity

1. INTRODUCTION

The living resources of our inland estuarine coastal and oceanic ecosystems are being harmed by extensive dumping of waste material and the runoff highly polluted waters. The rate of increase of pesticide and herbicide use is relatively high in India. In recent years, awareness about the potential harmful effects of the residues on human health has been growing (Saeed et al., 2005) and its contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Rahman et al., 2002).

Pesticides are also known to influence the freshwater ecosystem and contaminate different compartments of the ecosystem (Lindgaard–Jorgensen and Bender, 1994). Pesticides and herbicides at high concentrations are known to reduce the survival, growth and reproduction of fish (Rahman et al., 2002; McKim et al., 1975). The introduction of toxic chemicals causes toxic effects to organisms (Cope, 1965; Reddy and Rao, 1987) because these are liable to use rather heavily than normal doses required. This induces alterations in the metabolic cycle counteract threat induced by the stress conditions. The pathological and biochemical disturbance in aquatic organisms due to pesticide toxicity is well documented (Muley and Mane, 1990). These disturbances can be structural and functional at the cellular and sub cellular levels in organisms (Rodriquez et al., 1994).

Among pollutants herbicides are major contaminants in the lake Kondakarla (17° 36’ N; 83º0’ 0E) situated at a distance of 48 km from the Visakhapatnam, A.P., and East coast of India. A matter of deep concern is the pollution of lake in recent years. Around the lake there is extensive cultivation of rice and farmers extensively use the herbicides to control aquatic weeds. Paraquat (1, 1’-dimethyl – 4, 4’- bi pyridinium) is a common herbicide used by farmers to control weeds (Kimbrough, 1974; Calderbank 1975; Dasta, 1978; Hughes, 1988; Smith, 1988). In India it is available (both solid and liquid states) in the form of Gramoxone. It is a bipyridinium group of herbicide and its use in the aquatic habitat poses threat on aquatic organisms. Therefore, it is important to always determine the bioaccumulation capacity for pollutants by organisms’ especially aquatic ones, in order to assess potential risk to human health. The present study deals with the histopathological effect of the paraquat on the digestive gland of freshwater snail *L. luteola*.

2. MATERIALS AND METHODS

*Lymnaea luteola* is a freshwater snail commonly found in freshwater ponds, lakes where the water is stagnant with green vegetation such as Lotus, Hydrilla and some other aquatic plants. For this study lymnaeids were collected twice a week from the
Kumar and Priyadarsini
Histopathological Effect of Paraquat (Gramoxone) on the Digestive Gland of Freshwater Snail Lymnaea luteola (Lamarck: 1799) (Mollusca: Gastropoda)

Kondkarla lake situated at a distance of 50 km from the Visakhapatnam (East coast of India, 17°36’0N, 83°0’0E). This is a big natural Lake with hills on two sides and occupies an area of several square kilometers (1,100 hectares) and supports a rich variety of fauna and flora. They were picked by hand and maintained in the plastic container filled with pool water and transported to the laboratory and exposed to natural photoperiod. The water in the container was renewed once a day to provide freshwater rich in oxygen. Specimens were acclimatized to laboratory conditions in a glass aquarium for one week prior to experiment. For experimental purposes, active, healthy snails of equal groups were selected. Depending on the purity of the herbicide, required concentration was made by dissolving it in doubled distilled water. The commercial grade paraquat was diluted with double distilled water to prepare solution of tested concentrations. Preliminary screening tests were conducted to obtain the range of lethal concentrations and based on these, the experiments were carried out. For each concentration 10 acclimatized snails were selected in each aquarium containing different concentrations of herbicide as well as in the control described by Rahman et al (2002) respectively. Acute toxicity were performed under static conditions up to 96 hours by recommended methods (Committee, 1975) and median lethal concentration was computed using probit analysis (Finney, 1971). At the end of each exposure period, live animals were promptly removed from each exposure concentration and control. After the removal of the shell, tissues were fixed in Bouins alcohol and processed according to routine histological methods (Klobucar et al., 1997, 2001) dehydrated in alcohol and embedded in paraffin wax. They were cut into sections of 7-8µm thickness by rotary microtome. The sections were stained with different histological techniques for observation of histopathological changes in the digestive gland of L. luteola. In this analysis the percentage of mortality rates were converted into regression lines were drawn between log concentration and probit values. Each value represents the concentration ± standard error. The values in the parentheses represent 95% fiducial limits.

Table 1: Lethal concentration of Paraquat for Lymnaea luteola at 96 hrs

<table>
<thead>
<tr>
<th>Lethal Concentration</th>
<th>Concentration ppm (±)SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC 5</td>
<td>0.020 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>(0.016 - 0.024)</td>
</tr>
<tr>
<td>LC 10</td>
<td>0.026 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>(0.022 - 0.030)</td>
</tr>
<tr>
<td>LC 25</td>
<td>0.043 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>(0.038 - 0.048)</td>
</tr>
<tr>
<td>LC 50</td>
<td>0.073 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>(0.066 - 0.080)</td>
</tr>
<tr>
<td>LC 75</td>
<td>0.125 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>(0.112 - 0.138)</td>
</tr>
<tr>
<td>LC 90</td>
<td>0.203 ± 0.028</td>
</tr>
<tr>
<td></td>
<td>(0.175 - 0.231)</td>
</tr>
</tbody>
</table>

Footnotes: The values were calculated from the regression equation (Y = 2.505 + 2.885 X), correlation coefficient (r) = 0.927. Each value represents the concentration ± standard error. The values in the parentheses represent 95% fiducial limits.

3. RESULTS AND DISCUSSIONS

The histopathological alterations in the digestive gland of the freshwater snail L. luteola induced by paraquat were studied in the laboratory. Paraquat caused significant histopathological changes in the digestive gland of the snail based on its exposure period. Acute toxicity of series of concentration of paraquat of L. luteola was determined by recording the snail mortality at 24, 48, 72 and 96 hrs exposures. The LC50 value based on the probit analysis was found to be 0.073 ml/L for 96 hrs of exposure to the herbicide. Results obtained shown that there was no mortality either in control and exposed to 0.0196 ml/L paraquat throughout the 96 hrs 100% mortality was recorded with 48hrs, on exposed to 0.790 ppm concentration of stock solution of paraquat. The results shown that LC50 for 24 hrs 0.117 ppm and for 48 hrs 0.265 ppm while the safe concentration for 72 hrs 0.202 ppm and for 96 hrs 0.073 ppm (Table 1 and Fig.1).

Paraquat causes significant dose dependent histopathological changes in the digestive gland of L.
*Lymnaea luteola*. Normal digestive gland (Fig. 2) of *Lymnaea luteola* is a large tubule acinar gland which occupies the greater part of the cavity of the shell spiral. The gland is covered by squamous epithelium resting on a thin layer of fibrous connective tissue. The digestive gland tubules are lined with simple epithelium. This epithelium consists of two main cell types, digestive cells and secretory cells. The secretory cells are present in much smaller number than in the digestive cells they are shorter pyramidal or cone shaped but may sometimes be columnar. They are markedly shorter than the digestive cells and therefore appear wedged in between groups of the digestive cells. The histopathological changes induced accumulation of amebocytes in the hemolymphatic spaces between the tubules of digestive gland, exudation in the lumen of tubules, expansion of hemolymphatic spaces between the tubules and increase of vacuolation and necrotic changes in the digestive cells (Fig. 3-5). The digestive glands of molluscs have been known as target organs for contaminant effects because; this organ plays a major role in contaminant uptake, intracellular food digestion and metabolism of inorganic and organic chemicals in the organisms (Rainbow and Philips, 1993; Marigomez et al., 2002; Usheva et al., 2006).

![Graph](image1.png)

**Fig. 1:** Regression line representing the relation between probit values and Paraquat concentrations in *Lymnaea luteola* at 96 hrs

![Image](image2.png)

**Fig. 2:** Sagittal section of digestive gland (control) of *L. luteola*

![Image](image3.png)

**Fig. 3:** Sagittal section of digestive gland of *Lymnaea luteola* exposed to 0.0731 ml/L Paraquat for 48 hrs

CT - Connective Tissue; DT - Digestive Tubule; DGL - Digestive Gland Lumen; DAC - Digestive Absorptive cells; EC - Excretory Cell; A - Increase the secretion of digestive tubules
Kumar and Priyadarsini

Histopathological Effect of Paraquat (Gramoxone) on the Digestive Gland of Freshwater Snail *Lymnaea luteola* (Lamarck: 1799) (Mollusca: Gastropoda)

Fig. 4: Sagittal section of digestive gland of *Lymnaea luteola* exposed to 0.07311 ml/L Paraquat for 72 hrs
A- Degenerating cells; B- Exudation in the lumen of tubules

Fig. 5: Sagittal section of digestive gland of *Lymnaea luteola* exposed to 0.07311 ml/L Paraquat for 96 hrs
A- Increase of vacuolation; B- Cells completely necrotic.

The digestive gland is composed of numerous tubules and very small quantity of hemolymphatic spaces between the tubules was observed in each exposure of paraquat. Necrosis in the digestive cell and atrophy in the connective tissue were not seen in the snails maintained 24, 48 and 72 hrs, exposed to 2.29 to 0.073 ml/L of paraquat. However, these changes gradually increased in the snails in 96 hrs of exposure. The present results are comparable with the findings of Ot ludil et al. (2004) who reported that the histopathological examination after the exposure of ramshorn snail, *Planobarius corneus* to two sub lethal concentrations of Endosulfan for period of 10, 20 and 30 days revealed many changes such as degeneration of the cells of digestive glands, necrosis and atrophy in the digestive tubules and desquamation of the epithelial cells. Therefore necrotic damage in the epithelial cells may results in serious dysfunction of the tissue leading to deleterious effects at higher biological organization levels. Jonnalagadda and Rao (1996) have been reported histopathological alterations such as degeneration and the gathering of
amebocytes in areas between the tubules in the digestive gland of snail *Bellamya dissimilis* exposed to endosulfan, *P. corneus* exposed to PCP (Klobucar et al., 1997) and *Amphimelania holandri* exposed to phenol (Lajtner et al., 1996). Amebocytes are characteristic in acute inflammatory process. Rondelaud and Dreyfuss (1996) also showed that in snails (*Lymnaea glabra*) exposed to sub-lethal concentration of niclosamide group, histopathological lesions such as epithelial necrosis was observed in the digestive glands and gonads. The investigations of Unlu et al (2005) and Cengiz (2005) revealed that the histopathological effects of thiodan on two species of pulmonata snails, *Lymnaea stagnalis* and *Galba truncatula*. Rahman et al., (2002) observed the effect of Diazinon 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbolis gonicnotus Naga*. Omitoyin and Fajimi (2006) made some histopathological investigations on Juvenile African catfish, *Clarias gariepinus* exposed to non-selective herbicide gramozone (paraquat). The histopathological investigations of Nahlas El – Shenawy et al. (2009) also give more details on histopathological effects of different organs of Clam, *Ruditapes decussates*, exposed to organophosphorous pesticides and herbicides.

4. CONCLUSION

The present study has shown several degenerative changes in the histological structure of digestive gland of *L.luteola* exposed 0.0731ppm of paraquat concentration for 96hr. The digestive glands of molluscs have been known as target organ for contaminant effects because; this organ plays a major role in contaminant uptake, intracellular food digestion and metabolism of inorganic and organic chemicals in the organisms. Different concentrations of paraquat show 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 histopathological examinations reveal the degeneration of cells; secretory cells become irregular; necrosis of cells and atrophy in the connective tissue of digestive gland. The main targets of toxic action were lumen of the digestive gland tubule was shrunken, hypertrophied with conspicuous vacuolation in paraquat exposed to snails. All the histopathological observations indicated that exposure to sublethal concentration of paraquat caused destruction of tissue of *L.luteola*. Therefore indiscriminate use of paraquat by farmers should be discouraged particularly in area close to aquatic environment.

REFERENCES


Dr. K. Vijaya Kumar is an Assistant Professor and Head of the Department of Zoology in T.S.R and T.B.K PG College, affiliated to Andhra University. He had three years teaching experience in holding theory classes and four years’ experience in holding Lab sessions for M.Sc students in Cell and Molecular biology, Toxicology, Pathology and Molecular cytogenetic. He received his B.Sc., degree in Biological Science from Acharya Nagarjuna University and he obtained receive M.Sc, M.Phil and Ph.D (2010) in Zoology from Andhra University, Visakhapatnam. He has published many refereed articles in professional journals and proceedings. His area of specialization is Environmental Toxicology, Histopathology and Biochemical studies.

A.S. Priyadarsini is a lecturer and faculty member of St. Joseph’s College for Women (A), Gnanapuram, Visakhapatnam. She obtained M.Sc. Zoology from Andhra University in 2006. She has three years research experience in the field of histopathology and Toxicology. Presently she is a Ph.D student in the Department of Zoology, Andhra University, Visakhapatnam. She has special interest in the research area related to Toxicology and Environmental pollution.