ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

HISTOPATHOLOGICAL CHANGES CAUSED BY *PONGAMIA PINNATA* AQUEOUS LEAF EXTRACT IN THE GILL AND INTESTINE OF COMMON CARP- *CYPRINUS CARPIO*

SHOEIBA TASNEEM*, SYEDA HINA KAUSER, RAFATH YASMEEN

Department of Zoology, Osmania University College for Women, Koti, Hyderabad, Telangana, India.

Manuscript Info

Manuscript History:

Received: 15 September 2015
Final Accepted: 22 October 2015
Published Online: November 2015

Key words:

Biopesticidal, *Pongamia pinnata*, *Cyprinus carpio*, gill, intestine.

Corresponding Author

SHOEIBA TASNEEM

Abstract

Studying the toxicity of biopesticidal plant *Pongamia pinnata* aqueous leaf extract to common carp – *Cyprinus carpio*. 96 hr Lc50 value was 1300 ppm. The fishes were exposed to sub lethal concentration i.e., $1/4^{th}$ of Lc 50 value i.e., 350 ppm for a period of one week. After the completion of 24 hrs and 7 days the gill and intestine were dissected out, processed and sectioned at 4µm and stained with H & E stain. The slides were then observed under 40x magnification. The exposed gill showed histopathological changes such as distorted, shortened, twisted and curled secondary gill lamellae with some Vacuolation. The exposed intestine showed pathological changes such as Vacuolation of villi epithelium, lesions, rupture of epithelium and degeneration of villi epithelium. The pathological changes were more severe at 7days exposure in both gill and intestine.

INTRODUCTION

The continuous and indiscriminate use of synthetic pesticides for vector control in lakes, ponds and aquaculture farms has created the problem of acute and chronic toxicity to environment (Shafer; et al., 2005; Singh et., al., 2009). The synthetic pesticides due to their long-term persistence in water and fish body adversely affect the quality of fish and their status (Cullen and Connell, 1992; Waliszewski et.al, 1999). The aquatic ecosystem as a great part of the natural environment is also faced with the threat of a shrinkage genetic base and biodiversity due to indiscriminate use of pesticides (Omitoyin et al., 2006). To solve the problems, studies are being carried out on the feasibility of using biopesticides or plant extracts. Plants are virtually inexhaustible source of structurally diverse biologically active substances (Istvan, 2000).

Pongamia pinnata (Merr) has also been called *Derris indica; Pongamia glabra; Millettia pinnata* and belong to family: Leguminosae (ICFRE). It is one of the few nitrogen fixing trees and produce seeds containing 30-40% oil. It is often planted as an ornamental and shade tree. The species is commonly called as pongam; karanga or a derivation of these names. The plant contains some insecticidal properties (Anon, 1994).

Fishes are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution (Lopes et. al., 2001; Whitefield & Elliott, 2002 & Dautremepuits et. al., 2004). The common carp, *Cyprinus carpio* (Linn.) is the most extensively transplanted species of fish in the world, thus supplying essential sources of protein for the human food consumption. The fish is very much preferred for cultivation in ponds because of its excellent growth rate, omnivorous habit, breeding in confined water, hardy nature and easy adaptability to artificial feeds. The possibility of rearing carp in flooded rice fields has been contemplated for many years, superior rice fields were obtained from field in which carp was reared.

The present study involves the effect of *Pongamiapinnata* plant aqueous leaf extract on histological changes in gill and intestine of the common carp *Cyprinus carpio*.

Materials and methods:

Fish: The common carp; *Cyprinuscarpio* ranging in length from 10± 0.5cm and 8± 0.25grams in weight were collected from kolsagar reservoir; Mahaboobnagar; Telangana; India. The fishes were stocked in 500 litre tank having dechlorinated tap water and were acclimatised for 15 days. The fishes were fed twice daily with commercially available pellets. The water was renewed after 24 hrs daily.

Preparation of aqueous leaf extract: Mature leaves of plant *Pongamia pinnata* (Merr.), Family: Leguminosae, were collected from Osmania University campus, Hyderabad, Telangana, India. The leaves were thoroughly washed and dried in shade for 10 days and then pulverised to fine powder in an electric blender. 5% of aqueous leaf extract was prepared by dissolving 50 grams of powdered leaves in 1 lit of distilled water and kept at room temperature for 24 hrs. After 24 hrs the mixture was filtered and the extract was used immediately used in the experiment.

Determination of 96hr Lc50 and sub-lethal toxicity testing: The fishes were divided in 11 groups; each group having 10 fishes in glass aquaria having 15 litres of dechlorinated tap water. 24hrs before the commencement of Lc50 testing the fishes were stopped feeding. The concentration of *Pongamia pinnata* aqueous leaf extract in the aquaria was 800 ppm, 900 ppm, 1000 ppm, 1100 ppm, 1200 ppm, 1300 ppm, 1400 ppm, 1500 ppm, 1600 ppm and 1700 ppm respectively and the 11^{th} group served as the control. The aquaria were observed for 96 hrs to see the Lc50 value. Throughout the 96 hr Lc50 testing the fishes were observed for clinical signs like skin pigmentation, swimming pattern, response to stimuli and mortality. The 96 hr Lc50 value was recorded and tested by probit analysis as described by Finney (1971).

$1/4^{th}$ of the 96hr Lc50 value was taken as sub lethal concentration i.e., 350 ppm. 10 fishes were exposed to the sub lethal concentration for a period of one week. Throughout the exposure period the fishes were fed twice daily with commercial pellets and the water was renewed after 24 hrs. One group of fishes did not receive any concentration and it served as the control group. After the completion of 24 hrs and 7 days the fishes from both the exposed and control group were dissected and the gill and intestine were carefully removed and washed in 0.9% saline and fixed in 10% formalin for 24 hrs. The tissues were then dehydrated in graded series of ethanol, embedded in paraffin and sectioned at 4µm and stained with H & E stain. The slides were observed under light microscope at 40x magnification and were then photographed with Olympus digital camera attached to the microscope.

Results:

The 96 hrs lc50 value was 1300ppm and $1/4^{th}$ of 96 hr LC50 value has been taken as sub lethal concentration i.e., 350ppm. 10 fishes were exposed to the sub lethal concentration for a period of 7 days and at the end of 24hrs and 7 days the gill and intestine from both control and exposed group were dissected and examined at 40x magnification using H & E stain.

Sl. No.	Concentration of aqueous leaf extract in ppm	Number of fishes exposed	Number of fishes dead after 96 hrs
1.	0	10	0
2.	800	10	0
3.	900	10	0
4.	1000	10	1
5.	1100	10	3
6.	1200	10	4
7.	1300	10	5
8.	1400	10	6
9.	1500	10	8
10.	1600	10	10
11.	1700	10	10

GILL:

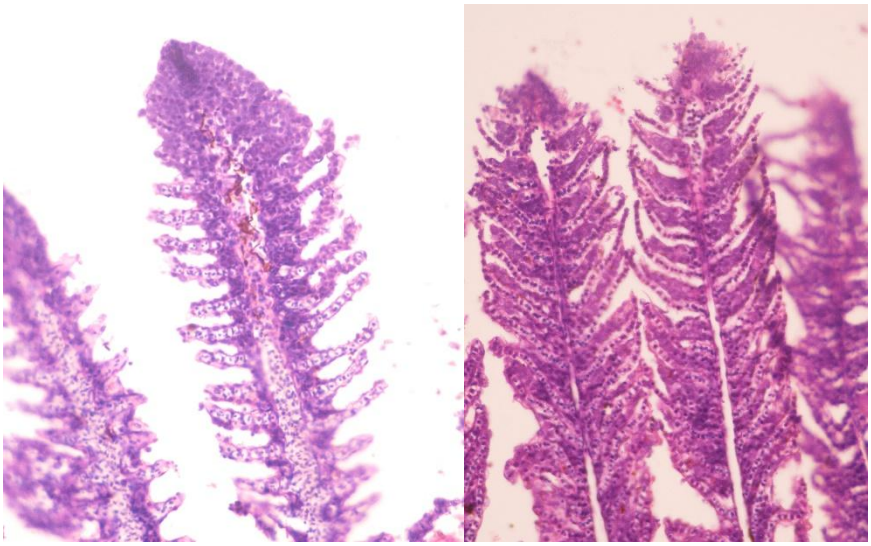

Fig: 1, Gill of control fish, 40x, H & E

Fig: 3, Gill of fish exposed to *P.pinnata* aqueous leaf extract for 7 days, 40x, H & E

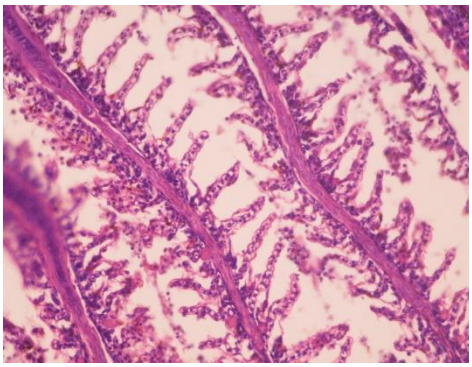

Fig: 2, Gill of the fish exposed to *P.pinnata* aqueous leaf extract for 24 hrs, 40x, H & E

The gill of the control fish shows the following structure **(fig: 1).** below operculum are present four branchial arches. Each branchial arch consists of two hemibranchs consisting of two rows of tapered and flattened gill filaments. On upper and lower surfaces of each gill filament are a series of flattened leaf like structures, each called as secondary gill lamellae, which form the respiratory surface. Epithelial wall of each secondary gill lamellae is held apart and supported by pillar cells. The gill exposed to the *Pongamia pinnata* aqueous leaf extract for 24hrs **(fig: 2)** showed the following pathological changes such as distorted and shortened secondary gill lamellae, twisted and curled secondary gill lamellae, cells of the secondary gill lamellae showed Vacuolation to some extent. The gill exposed to the Pongamia pinnata aqueous leaf extract for 7 days **(fig: 3)** showed the following alterations very narrow and elongated secondary gill lamellae, slight bending of the tips of the secondary gill lamellae, curling of secondary gill lamellae, Vacuolation of cells of secondary gill lamellae, slight rupture of respiratory epithelium, the cell mass between secondary gill lamellae became massive to such an extent that interlamellar spaces were completely occluded giving the gill filament compact appearance.

Intestine: The intestine of the control fish showed the following structure **(fig: 4)** serosa-outermost covering consisting of a single layer of epithelial cells, subserosa- smooth muscle fibres arranged in definite pattern the outer being longitudinal and inner circular, submucosa-consists of connective tissue fibres, nerves and blood vessels, muscularis mucosa-with two layers of muscles i.e., outer longitudinal and inner circular, gastric mucosa-epithelial

coat forming inner layer formed of columnar prismatic cells with basically located nuclei. The entire mucosa is folded into number of finger like process called villi. The intestine of the fish exposed to *Pongamia pinnata* aqueous leaf extract for 24 hrs **(fig: 5)** showed the changes such as inflammation at the base of the villi, vacuolation of cells of villi epithelium and compaction of the villi. The intestine of the fish exposed to *Pongamia pinnata* aqueous leaf extract for 7 days**(fig: 6)** showed the changes such as lesions in the villi, rupture of villi epithelium, massive infiltration of inflammatory cells throughout the villi and degeneration of villi epithelium.

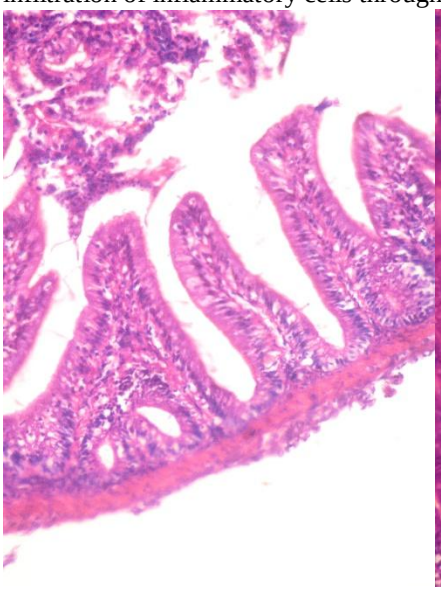

Fig: 4, Intestine of control fish, 40x, H & E

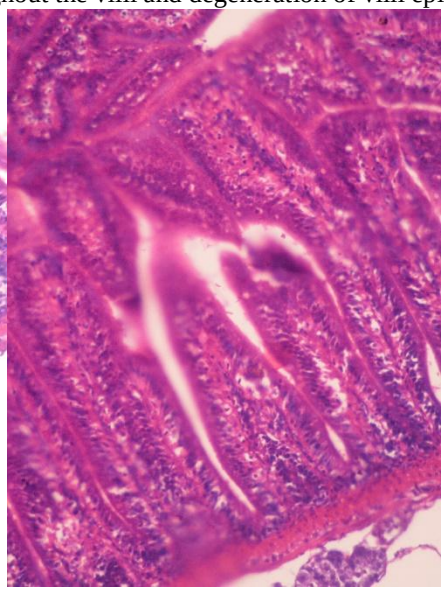

Fig: 6, Intestine of fish exposed to *P.pinnata* Aqueous leaf extract for & days, 40x, H 7 E

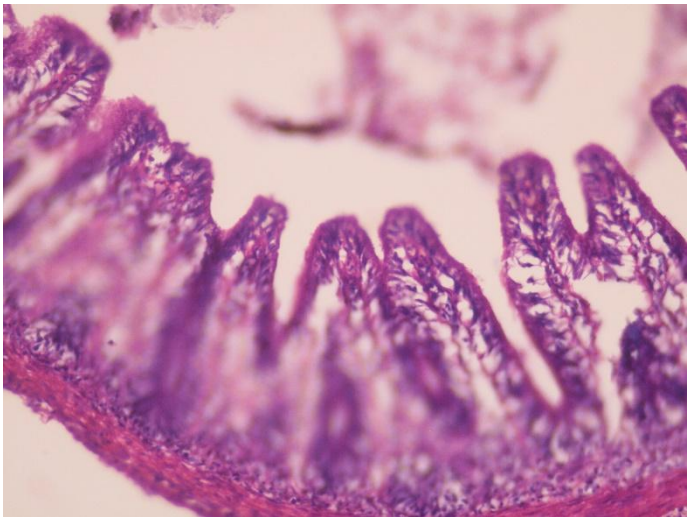

Fig: 5, Intestine of fish exposed to *P.pinnata* aqueous leaf extract for 24hrs, 40x, H & E

Discussions:

The purpose of the study was to evaluate the histopathological damages induced by sub lethal concentration of *Pongamia pinnata* aqueous leaf extract in the gill and intestine of common carp, *Cyprinus carpio*. Individuals in the control group did not display any histopathological changes in any of the examined tissues.

Fish gills have many important functions including exchange of gases, transport of many mono and divalent ions, excretion of waste nitrogen, uptake and excretion of various xenobiotics (Zayed & Mohamed 2004: Evans et al.,2005). Damage to gill tissue may interfere with gas exchange performance of gill and

cause respiratory disorders, ion-regulation and osmoregulation dysfunction and inefficacy of the excretion of waste nitrogen metabolite in exposed fish (Nero et al., 2006; Cengiz and Unlu, 2006; Velmurugan et al., 2007). The damage to the gill observed in the present study such as vacuolation of cells of secondary gill lamellae, bending and curling of tips of the secondary gill lamellae were also observed after exposure of mosquitofish (*Gambusia affinis*) to deltamethrin (Cengiz and Unlu, 2006), yellow perch and (*Perca flavescens*), goldfish (*Carassius auratus*) to oil sands (Nero et al., 2006), yellow perch (*Perca flavescens*) to naphthenic acid (Nero et al., 2006), carp (*Cyprinus carpio*) to deltamethrin (Cengiz, 2006), and rainbow trout (*Oncorhynchus mykiss*) to maneb and carbaryl (Boran et al., 2010) and gourami (*Trichogaster trichopterus*) to paraquat (Banaee et al., 2013).

Intestine plays a very important role in absorption of nutrients by the villi epithelium. In the present study the intestine showed the changes such as leisions and rupture of the villi epithelium, infiltration of inflammatory cells throughout the villi, such pathological alterations were seen in the intestine of many other fish species exposed to different kind of pollutants and pesticides.Necrosis, degeneration, and accumulation of lymphocyte in lamina propria were observed in the intestine of mosquitofish, *Gambusia affinis*, exposed to Thiodan and deltamethrin (Cengiz et al., 2001; Cengiz and Unlu, 2006) and *Cirrhinus mrigala* treated with lambdacyhalothrin (Velmurugan et al., 2007). This result is similar to the observations by Glover et al. (2007) in Atlantic salmon (*Salmo salar)* to dietary endosulfan exposure.Suchismita and Abhik Gupta(2013)have also observed similar pathological lesions in the intestine of Malathion treated *Esomus danricus.*

Conclusion:

The present study shows that *Pongamia pinnata* aqueous leaf extract is toxic to *Cyprinus carpio* and affects the structure and function of respiratory system and intestine at sub-lethal concentrations causing considerable deterioration of fish health. Therefore it is concluded that the use of *Pongamia pinnata* aqueous leaf extracts can be used as a biological control in eradicating predators and unwanted organisms in the pond by the farmer instead of using agrochemicals and also because of its toxicity its usage should be monitored well.

Acknowledgement:

Authors wish to thank Dr. C. Srinivasulu, (Ast. Professor, Dept. of Zoology, Osmania University) for providing the microscope and camera. We give our sincere thanks to the Histopathological division of NIN Hyderabad for providing training in histopathology. ST thanks UGC-MANF scheme for providing the fellowship.

References:

Anon. (1994). Biological control agents of social forests insects. Entomology Research Institute, Loyola College; Madras, India, 11p.

Banaee, M., Davoodi, M.H. and Zoheiri, F. (2013). Histopathological changes induced by paraquat on some tissues of gourami fish (*Trichogaster trichopterus*). Open Veterinary Journal, 3(1), 36-42.

Boran, H., Altinok, I. and Capkin, E. (2010). Histopathological changes induced by maneb and carbaryl on some tissues of rainbow trout, *Oncorhynchus mykiss*. Tissue Cell, 42 (3), 158-64.

Cengiz, E. I. (2006). Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environmental Toxicology and Pharmacology, 22, 200–204.

Cengiz, E. I., and Unlu, E. (2006). Sub-lethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. Environmental Toxicology and Pharmacology, 21, 246–253.

Cengiz, E. I., Unlu, E. and Balci, K. (2001). The histopathological effects of Thiodan® on the liver and gut of mosquito fish, *Gambusia affinis*. J. Environ. Sci. Health B., 36 (1), 75–85.

Cullen; M. C & Connell, D.W. (1992). Bioaccumulation of chlorohydrocarbon pesticide by fish in the natural environment, Chemosphere, 25: 1579- 1587.

Dautremepuits, C., Paris- Palacios, S., Betoulle, S. & Vernet, G., (2004). Modulation in hepatic & head kidney parameters of carp (*Cyprinus carpio*. Linn) induced by copper & chitosan. Com. Biochem. Physiol. C. Toxicol. Pharmacol, 137, 325- 333.

Evans, D. H., Piermarini, P. M. and Choe, K P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. Physiol. Rev., 85, 97–177.

Finney D. J., (1971). Probit Analysis, 3rd Edn. Cambridge University Press; London.

Glover, C. N., Petri, D., Tollefsen, K. E., Jørum, N., Handy, R. D. and Berntssen, M. H. G. (2007). Assessing the sensitivity of Atlantic salmon (*Salmo salar*) to dietary endosulfan exposure using tissue biochemistry and histology. Aquatic Toxicology, 84, 346–355.

ICFRE (Indian Council of Forestry Research & Education). Undated. *Pongamiapinnata*. Forest Research Institute; Dehradun; India. 12p.
Istvan, U. (2000). Semi- natural products & related substances as alleged botanical pesticides. Pest management Science. 56(8): 703-705.
Lopes, P. A., Pinheiro, T., Santos, M. C., da Luz Mathias, M., Collares- Perira, M. J. & Viegas- Crespo, A. M., (2001). Response of antioxidant enzymes in the freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Sci. Total. Environ. 280, 153- 163.
Nero, V., Farwell, A., Lee, L. E. J., Van Meer, T., MacKinnon, M. D. and Dixon, D. G. (2006). The effects of salinity on naphthenic acid toxicity to yellow perch: Gill and liver histopathology. Ecotoxicology and Environmental Safety, 65, 252–264.
Nero, V., Farwell, A., Lister, A., Van Der Kraak, G., Lee, L. E. J., Van Meer, T., MacKinnon, M. D., and Dixon, D. G. (2006). Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Ecotoxicology and Environmental Safety, 63, 365–377.
Omitoyin, B. O. Ajani, E. K. Adesina, B. T. & Okuagu, C. N. F. (2006). Toxicity of Lindane (Gamma Hexachloro-Cyclohexane) to *Clarius gariepinus* (Burchell 1822). World Journal of Zoology. 1(1): 57- 63.
Shafer, T. J., Meyer, D. A. & Crafton, K. M. (2005). Developmental neurotoxicity of pyrethroid insecticides: critical review & future research needs. Environ. Health Persp. 113: 123- 136.
Singh, P., Kumar, P., Singh V. K & Singh, D. K. (2009). Effect of snail attractant pellets containing plant molluscicides on certain enzymes in the nervous tissue of *Lymnea acuminata*. The Bioscan 4(3): 395- 398.
Suchismita Das and Abhik Gupta, 2013. Histopathological changes in the intestine of Indian flying barb (*Esomus danricus)* exposed to organophosphate pesticide, Malathion (EC 50) .J B.A.H.S., 2 (2):90-93.
Velmurugan, B., Selvanayagam, M., Cengiz, E. I., and Unlu, E. (2007). Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. Environmental Toxicology and Pharmacology, 24, 286–291.
Waliszewski, S. M., Aguirre, A. A., Benitz, A., Infanzon, R. M., Infanzon, R. & Rivera, J. (1999). Organochlorine pesticides residues in human blood serum of inhabitants of Veracr Mexico. Bulletin of environmental Contamination & toxicology, 62: 397- 402.
Whitefield, A. K. & Elliott, M., (2002). Fishes as indicators of environmental & ecological changes within estuaries; a review of progress & some suggestions for the future. J. Fish. Biol, 61 (1), 220- 250.
Zayed, A. E. and Mohamed, S. A. (2004). Morphological study on the gills of two species of fresh water fishes: *Oreochromis niloticus* and *Clarias gariepinus*. Ann. Anat., 186, 295-304.