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Research Article

Histopathological alterations in the kidney of Gambusiaaffinis after exposure to chlorpyrifos

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ABSTRACT

Chlorpyrifos, an organophosphate insecticide, was evaluated for its histopathological effects on fish *Gambusiaaffinis*, by light microscopy. Chlorpyrifos is a broad-spectrum organophosphate for agriculture. This study aimed to assess the histopathologicalchanges of kidney of fish *Gambusiaaffinis*. In the present study *Gambusiaaffinis* were exposed to $1/10^{th}$ of sub-lethal concentration ($1/10^{th}$ of LC_{50} value is 0.0284 ppm) of chlorpyrifos, (LC_{50} is 0.284 ppm) for a period of 15, 30 and 45 days. The treated fish groups were compared with the control group for the histopathological changes in tissues of kidney tissue and marked changes were observed. The renal tissues would be at major toxicological risk since they receive large volumes of blood flow from both the renal portal venous system and the renal arteries. The kidney showed vacuolated epithelial cells, shrinkage of uriniferous tubules, deformed tubules and hypertrophy with pyknotic nuclei of the cells in the renal tubules due to chlorpyrifos toxicity. These alterations were time and dose dependent.

Key words: Histopathological changes, *Gambusiaaffinis*, Chlorpyrifos and Kidney

Introduction

Insecticides have been reported to contaminatenot only surface water, but ground water, rainwaterand fog water all of which lead to the insecticidesbeing transported long distances across nationaland regional boundaries [1]. Due to rapidvolatilization property of many insecticides, theyhave the tendency to pollute air around areas ofapplication [2], and can travel by air to sites furtheraway from the site of application. Direct andindirect contamination of aquatic environment bypesticides may cause fish kills, reduce fishproductivity and elevate concentrations of undesirable

chemicals in fish tissues. Environmental monitoring of pesticides can be for the purpose of generating baseline information, assessing the effects of pesticides to the environment and or continuous measurement of environmental load to ensure that regulatory requirements and standards are being met [3].

Chlorpyrifos is an organophosphate insecticide. It is very highly toxic to freshwater fish, estuarine and marine organisms [4]. Typically exposures of about 3 ppm are lethal to fish [5]. Chlorpyrifos is directly toxic to the nervous system and is also transformed inside animals to chlorpyrifos-oxon and 3, 5, 6-trichloro-2-pyridinol (TCP) both of which are many times more toxic to the nervous system than chlorpyrifos itself [6]. The use of chlorpyrifos as a termiticide poses the greatest risk to non-target organisms in aquatic environments due to the fact that a high rate of application is required i.e. over 500kg/ha compared to less than 5kg/ha for crops. Histopathology, with a broad range of causes, is increasingly being used as indicators of environmentalstress since they provide a definite biological end-point of historical exposure [7].

Pesticides drained indirectly toward the aquatic ecosystem that will effect on organisms found in water mainly on fish. Pesticides are useful tools in agriculture, but their contribution to gradual degradation of the aquatic ecosystem cannot be ignored [8]. Due to the residual effect of pesticides, important organs such as liver, gill and kidney will be damage. Diazinon is highly toxic to fish. Histopathological changes are important to identify the environmental effects of chemicals especially pesticides.Histopathology is mainly directed to study the effect of chemicals on the structural components of the living system and the ways in which cells and tissues respond to injury. A chemical or a derivative actingdirectly on the cell or most frequently causes chemical cytotoxicity by altering its environment. The cells in turn respond histopathologically by degeneration, proliferation, inflammation and repair. Hence, it is useful to have an insight into histological analysis regarding the extent of damage of the tissue, kidney whenchlorpyrifos enters the body of fish *Gambusiaaffinis.*

Material and Methods

Gambusiaaffinis(Cyprinodontiform: Poeciliidae) weigh (0.5-1.0 g), length (3.0-4.5 cm) were collected from local pond in Ajmer and acclimatized to laboratory condition. Test chambers were glass aquaria of about 50 liter capacity. The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15, 30 and 45 days conditioning period at room temperature. The fish were fed with commercial pelleted food at least once a day during this period. Acclimatized fish were not fed 24-hr before the start of the tests.

Mortality of fishes was recorded in each group for 96 hr. The regression equations were established by using probit - mortality and log of concentration of pesticide and LC50 value was determined. The fish was exposed to $1/10^{th}$ of sub-lethal concentration (0.0284 ppm) of chlorpyrifos at room temperature for a period of 15, 30 and 45 days. Concentration of chlorpyrifos in fresh water fluctuate both seasonally and within shorter time intervals and in the absence of precise information adversely affected by particular level at certain time of years. In the present work the histopathological effects have been under taken. For histopathological studies fishes were sacrificed after 15, 30 and 45 days of exposure at different concentration of chlorpyrifos. The kidney tissue was dissected out. Tissue must undergo preparatory treatment before microtomy, the slides were stained with eosin for half a minute and then place in 95% alcohol again. The stain sections were place in xylene for 20 minutes, then clean and mounted in D.P.X. The kidney of the fish exposed to dose of chlorpyrifos at room temperature showed significant effects, the result of present work showed the pathological symptoms.

Observations

Histopathological observation in Kidney

Kidney is usually located in the retroperitoneal position up against the ventral aspect of the vertebral column. It consists of two main parts, Cortex and medulla, in fish kidney, cortex includes nephrons (Glomerulus and Bowman's capsule) and renal tubules. Medulla has collecting tubules. Kidney is surrounded by capsule. It is light to dark brown organ normally extending the length of the body cavity. The nephron is followed by the collecting duct and the ureter. The glomerular capsule has as outer fibrous layer and inner flattened epithelium. The capsular epithelium is continuous with the renal tubular epithelium. The kidney, specifically the trunk (posterior) kidney, is one of the more important excretory organs of teleost fish. The kidney maintains delicate osmotic balances between the fish and the environment. The lymphoid tissue of the head (anterior) kidney and inter-tubular tissue of the trunk kidney are haemopoietic in function.

The kidneys are organs of obvious critical, though varied, function in fish species. One would assume that the renal tissues would be at major toxicological risk since they receive large volumes of blood flow from both the renal portal venous system and the renal arteries. Histologically, the teleost kidney is composed of two parts: The renal parenchyma of which the functional unit is the nephron and the interstitial tissue which consists mainly of hematopoietic tissue. The nephron of freshwater teleost kidney is divided into the following parts: **(1)** renal corpuscle (Malpighian body) consisting of the glomerulus and Bowman's capsule **(2)** renal tubules divided into a neck segment, proximal convoluted tube (consisting of two segments P1 and P2), an intermediate segment and a distal convoluted tube[9]. In The present study histopathological examinations of kidney of fish *Gambusiaaffinis,* presented in Plate 1 for control fish kidney, Plate 2 for 15 days of exposure, Plate 3 for 30 days exposure and Plate 4 for 45 day of exposure at 0.0284 ppm (1/10th of LD50) concentration of chlorpyrifos.

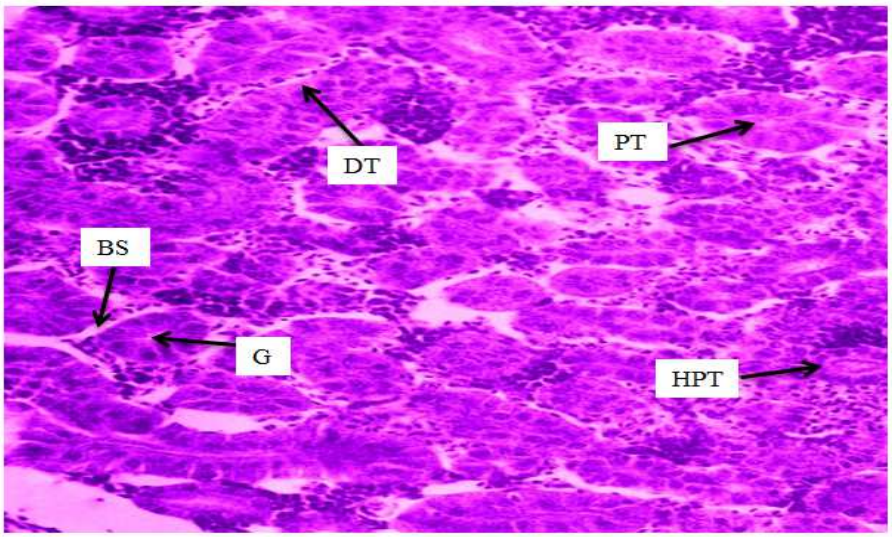

Plate-1: T.S. of Control Kidney showing BS: Bowman's space, DT: distal tuble, G: glomeule, HPT: hematopoitic tissue & PT: proximal tubule.

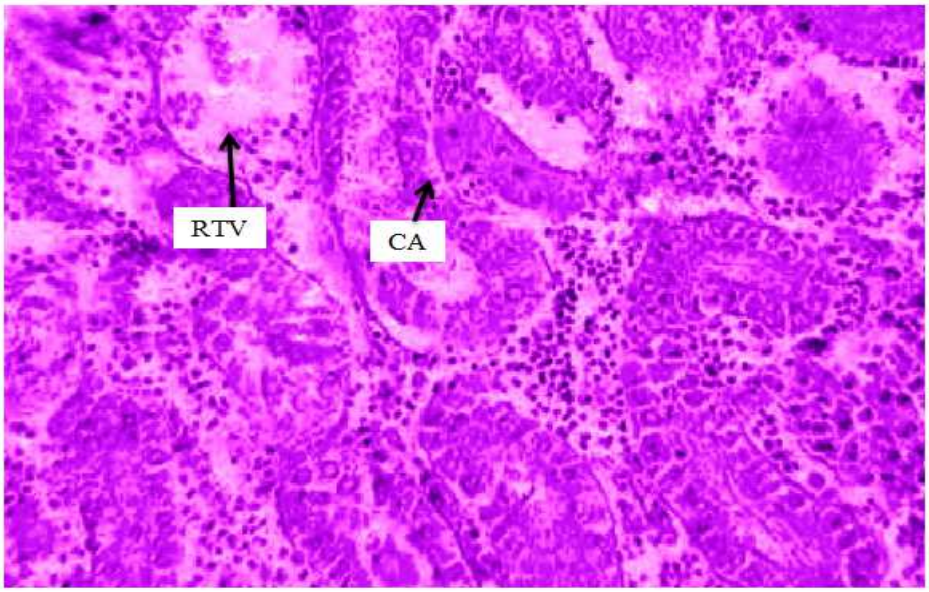

Plate-2: showed the epithelial cells of renal tubules are vacuolated (RTV) &clusters alteration (CA)on 15 days of exposure at 0.0284 ppm concentration of chlorpyrifos.

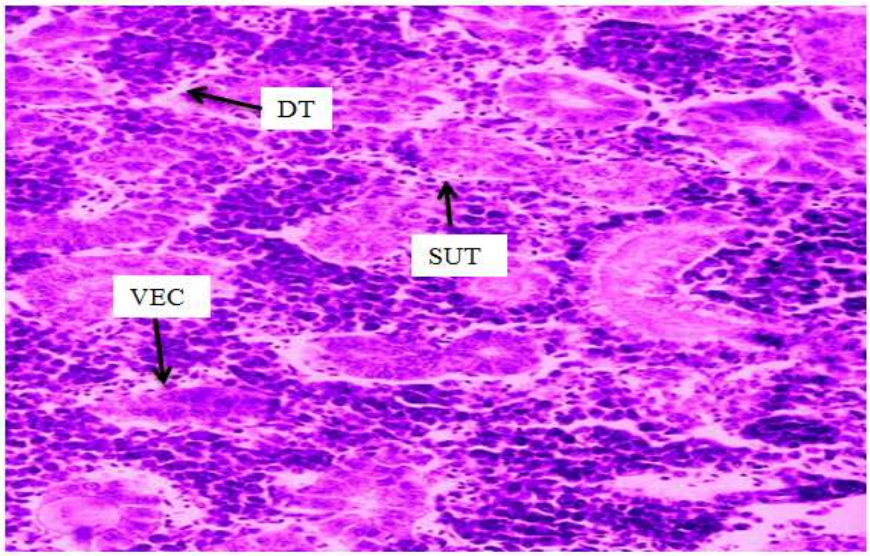

Plate-3: Showed Shrinkage of uriniferous tubules (SUT), deformed tubules (DT) &vacuolation epithelial cells on30 days of exposure at 0.0284 ppm concentration of chlorpyrifos.

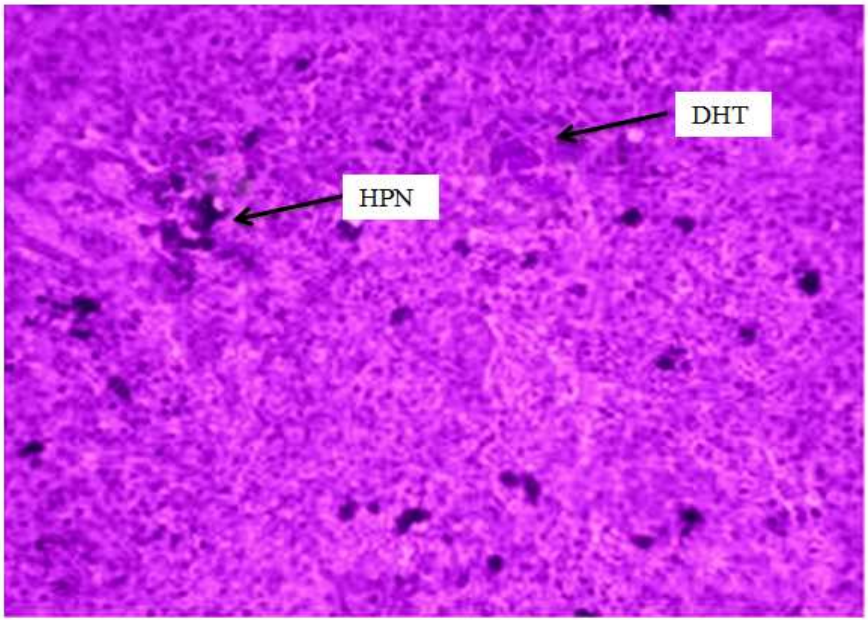

Plate-4: Showed beginning of degeneration in haemopoietic tissue (DHT) & hypertrophy with pyknotic nuclei (HPN) on 45 days of exposure at 0.0284 ppm concentration of chlorpyrifos.

Result and Disscussion

The kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20% of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of chemical in plasma could become toxic in the kidney. The kidney of the fish receives largest proportion of post branchial blood and therefore renal lesions might be expected to be good indicators of environmental pollution [10]. In the present study the most evident changes observed in the kidney of chlorpyrifos treated fish.

The kidney of control fish anatomy consists of glomerulus which is a tuft of capillaries, proximal tubules were characterized by columnar cells with brush border located along the apices of the cells while distal tubules were low columnar epithelium cells with basally round nucleus (Plate 1). Kidney of fish exposed to 0.0284 ppm concentration of chlorpyrifos for 15 days of exposure showed the epithelial cells of renal tubules are vacuolated (Plate2). These histological alterations consisted mainly of clusters alteration and vacuolation of tubular epithelial cells. Kidney of fish exposed to 0.0284 ppm concentration of chlorpyrifos for 30 days of exposure showedShrinkage of uriniferous tubules, deformed tubules and alteration of clusters and vacuolation of epithelial cells became very prominent (Plate3). Kidney of fish exposed to 0.0284 ppm concentration of chlorpyrifos for 45 days of exposure showed beginning of degeneration in haemopoietic tissue and hypertrophy with pyknotic nuclei in the renal tubules (Plate 4). The kidney plays the major role in this fight, producing large quantities of diluted urine. Although the kidney does not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in the liver have been shown to occur in the kidney. It receives the bulk of the post branchial blood flow; kidney tissue is of importance in the detoxification and elimination of aquatic contaminants in fish[11]. The kidney appears to be particularly sensitive to a variety of toxins due to the high renal blood flow, the ability to concentrate substances, and the biotransformation of the parent compound to a toxic metabolite [12].Cengiz observed lesions in the kidney tissues of the fish exposed to deltamethrin, characterized by degeneration in the epithelial cells of renal tubule, pyknoticnuclie in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intra cytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen [13]. Khan, S. studied Effect of cadmium chloride on histopathology of kidney of *Lebistes reticulates* and observed vacuolated epithelial cells in the renal tubule. [14] The Kidney showed shrinkage of uriniferous tubules and beginning of degeneration in haemopoietic tissue. The haemopoietic tissue showed necrosis and fibrosis.

Conclusion

The present study revealed a visual evidence of sterical derangement in kidney tissues of *Gabusiaaffinis*on 15, 30 and 45 days of exposure at different concentration of chlorpyrifos. The impairment of the cellular antioxidant defense or increases in the production of highly reactive free radical species may be the primary cause of cellular injury. Thereforeorganophosphorus insecticide chlorpyrifos is potent to cause toxic responses, even structural alterations, in aquatic organism like fish.

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