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RESEARCH ARTICLE

STUDY OF HISTOLOGICAL CHANGES IN KIDNEY OF *Clarias batrachus* (LINN. 1758) UNDER THE STRESS OF CYPERMETHRIN.

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Abstract

This study was conducted to assess the histopathological damage of kidney in *Clarias batrachus* after the exposure to cypermethrin. 25 fishes were selected and divided in to 5 groups, one (group I) is control and 4 (II,III,IV and V group) are treated. Fishes of treated groups were exposed to the sublethal concentration of 0.07 µl of cypermethrin for 1, 5, 10 and 15 days respectively. The kidneys of fishes were removed for histological examination. Lesions in the kidney tissues of fishes exposed to cypermethrin were characterized by degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, shrinkage of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and dialation of the tubular lumen. The results showed that the degree of distortion of kidney was proportional to the exposure periods and concentration of the cypermethrin.

Introduction:-

A variety of human activities such as industrial, agricultural and domestic has brought major changes in the aquatic ecosystem. Agricultural run-off laden with excess fertilizers and pesticides, industrial effluents with toxic substances and sewage water with human and animal wastes pollute our water. Untreated or improperly treated waste is a major cause of pollution of rivers and environmental degradation. One of the important pollutant that of pesticides, are chemical substances used to kill, repel or control certain forms of plants or animal life that are considered to be pests. The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and others. The organophosphates, organochlorine and pyrethroid pesticides are used to control agricultural pests as well as parasites in fishes but the toxicity of these pesticides to non-target organisms has been reported to be of great concern and their extensive use, pose a constant threat to aquatic life by altering the habitat behavior pattern, growth and reproductive potential. Compared to other pyrethroids, cypermethrin is relatively stable and is considered to be moderately toxic and like all pyrethroids, affects the central nervous system. Cypermethrin (CY), a synthetic pyrethroid insecticide has contact and stomach action properties which are extremely toxic to fish. Cypermethrin is metabolized and eliminated more slowly by fishes than by birds and mammals, which may explain this compound's higher toxicity in fish than in other vertebrates. It is a unique insecticide used mainly as residual treatment for the control of flies, mosquitoes and

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cockroaches. Though the terrestrial agriculture is the major source of food materials to mankind but supplementary proteins are needed in order to meet the prevailing protein deficiency. In many countries, the marine and freshwater fish serve as preferred animal proteins. The fish are the cheapest source of animal proteins to humans (Chakraborty *et. al.,* 2014). The walking catfish (*Clarias batrachus*) is a species of freshwater air breathing catfish native to Southeast Asia, but also introduced outside its native range where it is considered an invasive species. This catfish has long based dorsal and anal fins as well as several pairs of sensory barbels. The skin is scaleless, but covered with mucus, which protects the fish when it is out of water. When pesticides like cypermethrin enters into the body of fish, they damage and weaken the metabolism, leading to physiological, pathological, and biochemical disorders. The toxicity of pyrethroids for the fish has been assessed by several investigators (Begum, 2007; Singh *et. al.,* 2010; Raksheskar G.A., 2012,). Histopathological condition of fishes is affected to large extent when exposed to toxic substance. Thus the effect of cypermethrin on the histopathology of kidney of *Clarias batrachus* is so vivid because:- The kidney serves as a major route of excretion of metabolites of xenobiotics and is the main organ which balances the body fluids. Hence, it is useful to have an insight into histological analysis regarding the extent of damage of the kidney when cypermethrin enters the body of *Clarias batrachus.* Currently, the histopathological biomarker is one useful tool that can indicate environmental pollutants, especially for sub-lethal and chronic effects (Fatma, 2009; Nikalje *et. al.,* 2012).

Materials and methods:-

Experimental animal:-

Fresh water fishes (*Clarias batrachus*) were collected from government hatchery in dehradun with the help of fisherman. Fishes were brought to laboratory into aerated polythene bags filled with water and immediately given 0.05% potassium permanganate treatment for two minutes for disinfecting them. After disinfectant treatment fishes were transferred to the plastic tanks of 100 liter capacity with aerated tap water and were fed with commercially pellets. Fishes were acclimatized for two weeks under natural photoperiod and an average temperature of 25˚C. The tap water used for the experiment had a pH value of 7±0.1 and a total hardness of 20 mg CaCO3/L and was replaced every 4 days.

Experimental chemical:-

The commercial grade cypermethrin was purchased from market. 96 hr LC50 value for cypermethrin was determined by Finney's probit method and found to be 0.7µl/l.

Experimental set up:-

25 fishes (average weight of 15 gm and length of 12 cm) were finally selected and divided into five numerically equal groups I, II, III, IV and Vth. Group I served as control group and group II, III, IV and V were exposed to sub-lethal concentrations (0.07µl/l) of cypermethrin for 1, 5, 10, 15 days respectively. Fishes were sacrificed at 1^{st}, 5^{th}, 10^{th} and 15^{th} day after exposure. Fishes were dissected out carefully and kidneys were removed and fixed in bouins fluid for 24 hr and then processed and embedded in paraffin for block prepration. The sections were cut at 5-6 micron and stained in haematoxylin and eosine. The slides were examined under light microscope and photographed to observe the histopathological effects.

Result and Discussion:-

The primary function of the kidney in fish is the osmotic regulation of water and salts rather than the excretion of nitrogenous wastes as in mammals. In fish, the kidney must conserve salt and eliminate excess water. This is accomplished by a high glomerular filtration rate, reabsorption of salts in the proximal tubules, and dilution of urine in the distal convoluted tubule. The kidney is one of the first organs to be affected by contaminants in the water (Thophon, S. *et. al.,* 2003). Acute and chronic toxicity tests are mostly used to assess the toxicity of chemicals on non-target organisms (Santos *et. al.,* 2010). 96 h LC50 is one of the most important parameters for evaluating the toxic effects of pollutants (Nwani *et. al.,* 2015).

The histology of *Clarias batrachus* kidney in control fish is shown in the (Fig:1). In normal kidney architecture, the glomerular tissue was closely arranged with renal tubules including distal and collecting tubules and intact interstitial cells. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei. After I day treatment the changes in kidney histology were not very conspicuous, only mild changes were observed (Fig:2). Histological changes after 5 days treatment was shrinkage of glomerulus which

causes increase in space between glomerulus and bowmann's capsule, mild tubular degeneration with intact nuclei and cells. In some tubules, however the diameter of lumen is increased and nuclei of some epithelial cells become pycnotic (Fig:3).

After 10 days of exposure the tubuler lumen was remarkably widened, degenerative changes in tubular epithelium become enormous, vacuolization, nuclei degeneration and pycnotic nuclei was observed (Fig:4). These all changes became more severe after 15 days treatment and damaged blood vessels, hyaline degeneration of tubular epithelium, necrosis, pycnosis and shrinked glomerulus were observed (Fig: 5). Histopathological changes in kidney at level of glomerulus and tubular epithelium after exposure to pesticides have been reported by many other workers (Das and Mukherjee, 2000; Velmurugan *et. al.*, 2007, Gowri *et. al.*, 2013).

Cypermethrin exposure induced marked abnormalities in the kidney initiated with disruption of tubular organization. Thereafter degeneration of tubular epithelial cells and lympocytic infiltration, cloudy swelling of renal tubules in acute exposure was evident which can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm (Neelima *et. al.*, 2015).These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle. In more severe cases, the degenerative process can lead to tissue necrosis. . Histopathological changes in the kidney tissue such as severe necrosis, cloudy swelling, cellular hypertrophy and granular cytoplasm were reported by (Tilak *et. al.*, 2007) in *Channa punctatus* exposed to sublethal concentration of Butachlor and Machete, an Herbicide.

All the histopathological observation indicated that exposure to sublethal concentrations of cypermethrin caused destructive effect in the kidney tissues of *Clarias batrachus*. Kidney histopathological alterations, such as those observed in these studies and findings from previous studies could result in severe physiological problems, ultimately leading to the death of fishes.

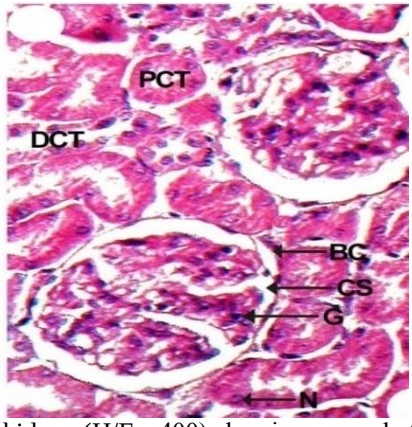

Fig: 1 Micrograph of control kidney (H/E ×400) showing normal structure of Glomerulus (G), Bowman's capsule (BC), Capsular space(CS), Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT), Nucleus (N).

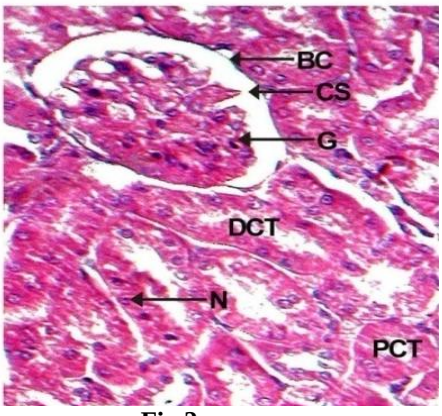

Fig 2

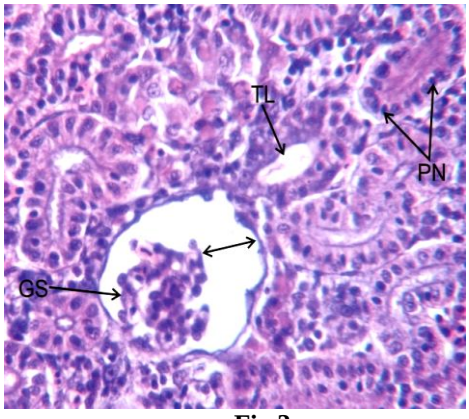

Fig 3

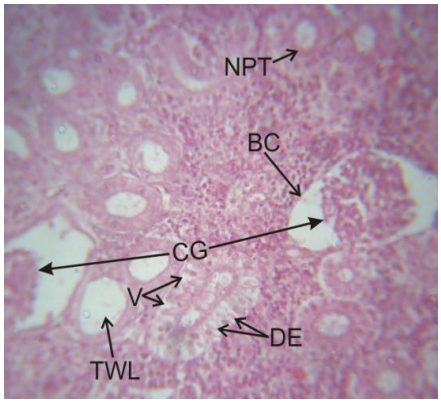

Fig 4

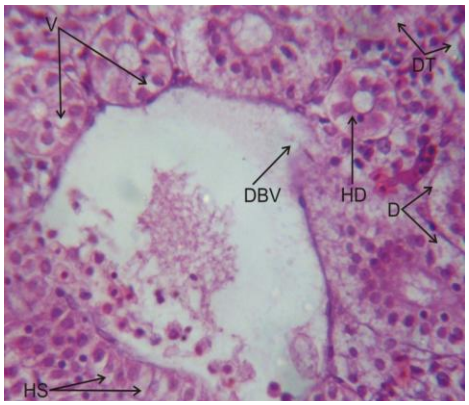

Fig 5

Fig 2:- Micrograph of kidney after 1 day showing mild damage in Glomerulus (G), Bowman's capsule (BC), Capsular space (CS), Proximal Convoluted Tubule (PCT) and Distal Convoluted Tubule (DCT).
Fig 3:- Micrograph of kidney after 5 days showing Glomerular shrinkage (GS), increase in space between glomerulus and bowman's capsule (↔), increased tubular lumen (TL), Pycnotic nuclei (PN) and relatively intact tubule.
Fig 4:- Micrograph of kidney after 10 days showing collapsing glomeruli (CG), Necrotic proximal tubules (NPT), Tubules with widened lumen (TWL), Degenerating tubules (DT), Vacuolized (V) and indistinct necrotic cells.
Fig 5:- Micrograph of kidney after 15 days showing Disorganized Tubules (DT), Hydropic swelling (HS), Vacuolization (V), Hyaline degeneration of tubular epithelium (HD), Desquamation (D) and Damaged blood vessels (DBV).

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