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HISTOLOGICAL CHANGES IN THE GILL AND LIVER OF FRESH WATER FISH *CHANNA STRIATUS* ON EXPOSURE TO PROPARGITE

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Abstract:
Study was designed to evaluate to assess the histology effects of propargite insecticides were treated on the gill and liver tissues of the fresh water fish, Channa striatus were determined by UV- Fluorescence microscopy. The effects of propargite were studied to determine the 96 h LC_{50}value (0.34ppm) on Channa striatus and investigate histology changes of fish exposed to sub- lethal concentration as control, 0.034ppm, 0.102ppm of 15days and 30 days of propargite. Observation of the gills and liver tissues results showed that remarkable effects of propargite toxicity as compared to control groups. However, histology damage in the gill and liver of the fish. The most common gill changes of propargite such as degenerated primary gill lamella, lamellar fusion, cellular necrosis and epithelial rupture, curling of secondary gill lamella, degenerate blood congestion, epithelial lifting, damaged epithelial cells, were reported. The liver appearance of blood streaks hepatocytes, close to a bile duct, degeneration vacuolar, pyknotic nucleic and congestion of sinusoids with necrosis in the treated fish. In conclusion, modify observed propargite is harmful to channa striatus at sub lethal concentrations and the pesticide close to bodies of water is a dangerous threat to aquatic life.

Keywords: *sub-lethal concentration, propargite, Histological, Channa striatus.*

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INTRODUCTION:

Restrictions with increasing on the use of organosulphuric pesticide compounds in the environment, organophosphates and carbamates have become the most commonly used pesticides in many parts of the world. However, routine applications of organophosphate pesticides may adversely affect many non-target organisms, such as fish. Propargite [2-(4-tert-butyl phenoxy) cyclohexyl prop-2-ynyl sulphite, is a non-systemic [1]. .An organosulphuric pesticide is a contact insecticide and selective acaracide, also used as a vector control agent for malaria in public health programs. Propargite is usually sprayed aerially. The pesticide may drift near rivers and ponds and creating potential danger for non-target organisms [2]. Although propargite is not very soluble in water, it has potential for surface water contamination by binding to and slowing dissipation from soil and sediment [3]. Although it is increasingly decline in most industrialized countries, still it is being used in tropical and sub tropical region, causing health of fish, water and food in various part of the world. Elevated residue levels of propagate in plant ingredients have also been reported [4].

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from pests. However, they are the major cause of concern for aquatic environment due to their toxicity, persistency and tendency to accumulate in the organisms [5]. Pesticides are generally used in contemporary agriculture to aid in the manufacture of high quality food [6]. Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non target organisms' are significant contamination of surface waters [7]. In agriculture[8]. Random use of different pesticides to prevent the crop from pest peril has increased in the developing countries the pesticides, even when applied to restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and change the water chemistry [9].

It may be highly toxic, not only to fishes but also to other organisms, including man [10]. The pesticides sold to them and unwittingly introduce dangerous chemicals into the environment [11]. When croplands are treated, some impacts of pesticides occur on non-target terrestrial and aquatic ecosystems, as well as on adjoining agro ecosystems [12]. The experimental species selected for the present investigation is *Channa striatus* and it is an ideal candidate for water toxicity studies. Striped or snakehead murrel, *Channa striatus*, is a commercially important species in Asia–Pacific region due to its tasty flesh, nutritional and medicinal properties [13]. Recently, we established laboratory-based hypoxia-stress-treatment protocol of the prolonged period in *C. striatus* [14]. Such experiment was in line with the fact that *C. striatus* is an air breathing fish that inhabits oxygen (O2) deficient muddy and marshy water, including the hibernation by burrowing in soft mud or under hard mud crust, to survive temporary drought [15].

Although toxicant impairs the metabolic and the physiological activities of the organism physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence, it is useful to have an insight into histological analysis, as they act as biological markers to assess the toxicity condition [16]. This stress typically disrupts organ physiology and metabolic processes. Thus, histology is an effective means of evaluating the health of fish exposed to contaminants and, more broadly, in assessing the extent of pollution in the tropic web [17, 18, 19].

The hematological parameters have been considered as diagnostic indices of pathological conditions in animals and could be used as potential biomarkers of insecticides [20, 21].
Histological techniques are sensitive, rapid, comparatively inexpensive tools and reliable for the assessment of stress-response of dietary ingredients. So when fish are exposed to environmental pollutants, these vital functions are deleteriously affected and the functional impairment of gills can significantly damage the health of fish. Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic process of fish. Many investigators have reported the histopathological changes in the gills of different fish species exposed to pesticides gill is the main osmoregulation organ in fishes, and it is highly sensitive to many factors, including stress, pollution and changes in the salinity of environment these toxic chemicals may persist in the environment for longer periods and show damage at histological level [22].

Liver is known among the most critical for facilitating hypoxia adaptation in fish species [23, 24]. Reported severe histological changes in liver of the fish Ophiocoephalus punctatus exposed to Malathion at different concentrations. Including gills and liver that are responsible for vital function such as respiration, excreation and the accumulation and biotransformation of xenobiotics in the fish [25].

view of the above, it was felt that it would be worthwhile to study the histological changes in liver of fish which would throw a clear light on the extent of effect that it causes. Hence in the present work, we studied the toxic effects of propargite on histological changes in gill and liver of fish, *Channa striatus*.

MATERIALS AND METHODS:

Chemical and Experimental design

The insecticide used in this experiment was propargite 57% EC organosulfite 2-(4-tert-butyl phenoxy) cyclohexyl prop-2-ynyl ester was purchased from Thanjavur, Tamilnadu, India. The propargite insecticide was used only for the present experiment. The experimental group was vulnerable to lethal concentration of the insecticide (0.34ppmL-1) during 15 and 30 days. Toxicity tests carried out in accordance with in standard methods [26]. A stock solution of propargite with a concentration of 1g per liter (equivalent to 1 ppm) was prepared in distilled water and different dilutions were prepared by adding the required amount of distilled water. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were fixed after conducting the range-finding test. The fishes were starved for 24 hours prior to their use in experiments as recommended by storage, to avoid any interference in the toxicity of pesticides by excretory products. After the addition of the toxicant into the test tank with 10 liters of water having twenty fish, mortality was recorded after 24, 48, 72 and 96 hours. Five replicates were maintained simultaneously.

Fish collection and laboratory conditions

The freshwater healthy fish *Channa striatus* of the weight (22.34±0.79g) and length (17 to 20cm) were selected for the experiment and were collected from ponds in and around Thanjavur. Fish was screened for any pathogenic infections. A Glass aquarium was washed with 1% KMnO4 to avoid fungal contamination and then sun-dried. The fishes were maintained in 300 L tank containing dechlorinated tap water (Temperature 26°C). Fish was acclimated to laboratory conditions for 15 to 30 days prior to experimentation. They were regularly fed with commercial food and the medium (tap water) was changed daily to remove fasces and food remnants.

Sub-lethal concentration

Based on acute toxicity test 96h LC50 (0.34ppm) sub-lethal concentrations (0.034ppm and 0.102ppm of 15 & 30 days) were derived from propargite which served as the experimental concentration of the propargite in the subsequent experiments. Ten fish were exposed to each groups for a period of 15 and 30 days. Control batch was maintained simultaneously.

Histological changes

For histological examination fish were sacrificed and tissues (gills and liver) were removed immediately to overcome autolysis. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution (75 ml saturated picric acid, 25 ml formaldehyde (37–40%) and 5 ml glacial acetic acid) for 48 h. Subsequently gill and liver

tissues were processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax block using. Gills and liver were processed by double embedding technique. Sections were cut at 4–6 _m thickness with the help of 820-Spencer rotator microtome, stained with hematoxylin–eosin (dissolved in 70% alcohol) [27]. The photographs at 100×magnification were taken with compound microscope with UV-Fluorescence [54].

Statistical Analysis

All the dates were subjected to one way ANOVA using statistical software of SPSS version 16.0. Duncan's Multiple Range test was used to determine the difference among treatment means at 5% level of significance.

RESULTS:

The present study histology changes of gills and liver of *Channa striatus* were investigated after propargite exposure by hematoxylin-eosin staining. Histological examinations indicate that propargite exposure affected the structural integrity.

Gills

Control gill tissues; No histology changes were observed in the gill of the control fish. The structural details C. *striatus* are shown in The normal architecture of the control fish gill sowing a primary gill lamella (PGL), secondary gill lamella (SGL), blood congestion (BC), pillar cell (PC) and erythrocyte (EC) (Fig -1)

Treated gill tissues

Gill histological changes the control fish revealed the intact nature of propargite 0.034ppm 15-30 days and 0.102ppm 15-30 days of exposure; further swelling of tip of secondary gill lamellae and their erosion were observed. Degenerated primary gill lamella (DPL), lamellar fusion (LF), secondary gill lamellae releasing blood cells was also seen at some places. Dilation in blood vessels of gill filaments (Fig- 2, 3).

These changes included the liver were more pronounced after 0.102ppm 15-30 days high exposure of propargite showing extensive cellular

necrosis and epithelial rupture (CNE), curling of secondary gill lamella (CSGL), degenerate blood congestion (DBC), epithelial lifting (EL), damaged epithelial cells(DEC), and dilation of sinusoid with blood congestion. Epithelium hyperplasia and fusion of the secondary lamellae, edema , Degeneration of pillar cells was observed aneurism, abnormal raising and swelling of the epithelium and excessive mucus secretion was observed (Fig 4,5).

Liver

Control Liver tissues

No histopathological changes were observed in the liver of the control fish. The structural details of the liver C. *striatus* are shown in Necrosis (N) hepatocytic cells (HC) blood vessels (BV) central vein (CV) vacuolar degeneration (VD) nucleus like structure (Fig- 6)

Treated liver tissues

The histology changes of liver exposed to propargite under sub lethal concentration 0.034ppm and 0.102ppm 15-30 days in the present study on liver indicates, propargite induced discrete histology changes in the liver tissues of *Channa striatus*. Control fish showing characterized hepatocytes with normal structure of liver (Fig- 7, 8).

More pronounced after 0.102ppm15- 30 days high exposure of propargite exposure period. These changes included the degeneration of cytoplasm in hepatocytes, pancerocyte, branchial epithelium, liver cell were irregular in shape and showed changes in nuclear and cell size *Channa striatus*. Appearance of blood streaks hepatocytes (ABS), close to a bile duct (BD), degeneration vacuolar (VD), pyknotic nucleic (PN) and congestion of sinusoids (CS). Among the sub-lethal concentrations of propargite this caused more severity to the liver structure was observed (Fig-9, 10).

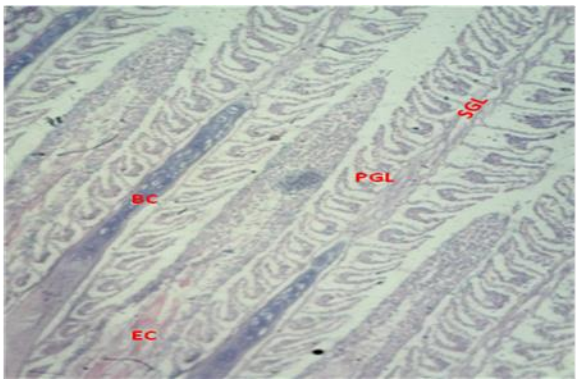

Fig-1: Gill tissue of *Channa striatus* fish control group: (H&E 10X)

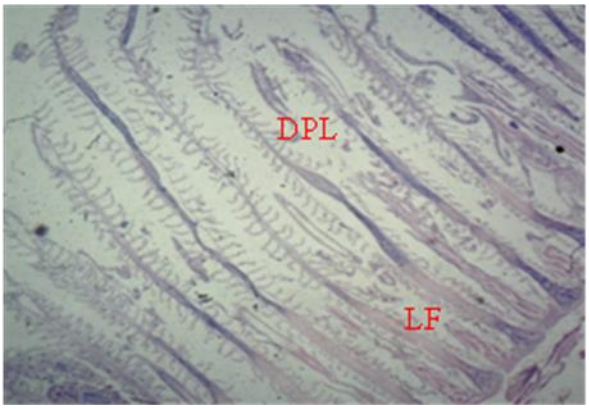

Fig-2: Gill tissue of *Channa striatus* fish exposed to15days sub lethal concentration propargite 0.034ppm (H&E 40X).

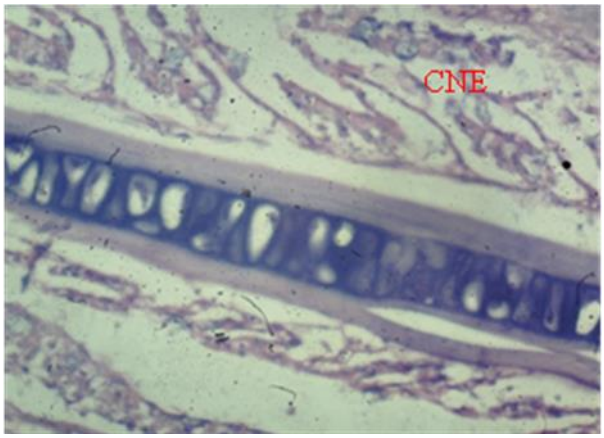

Fig-3: Gill tissue of *Channa striatus* fish exposed to30 days sub lethal concentration propargite 0.034ppm (H&E 40X).

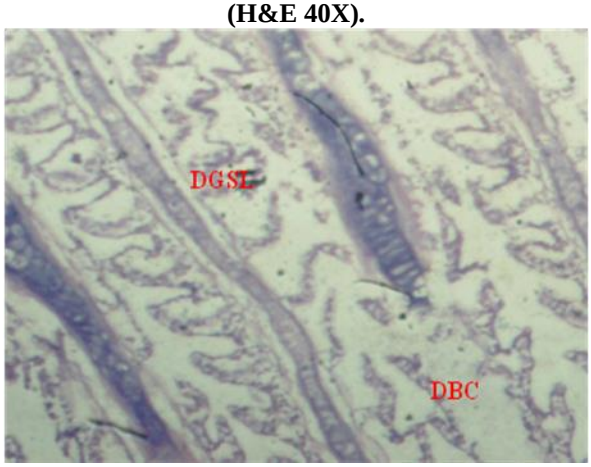

Fig-4: Gill tissue of *Channa striatus* fish exposed to15 days sub lethal concentration propargite 0.102ppm (H&E 40X).

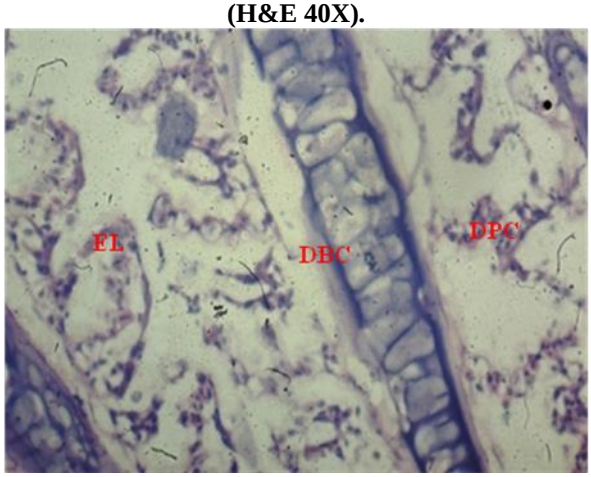

Fig-5: Gill tissue of *Channa striatus* fish exposed to 30 days sub lethal concentration propargite 0.102ppm (H&E 40X).

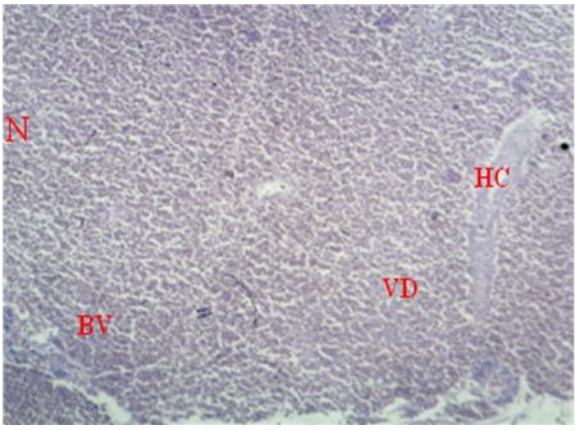

Fig-6: Liver tissue of *Channa striatus* fish control group: (H&E 10X).

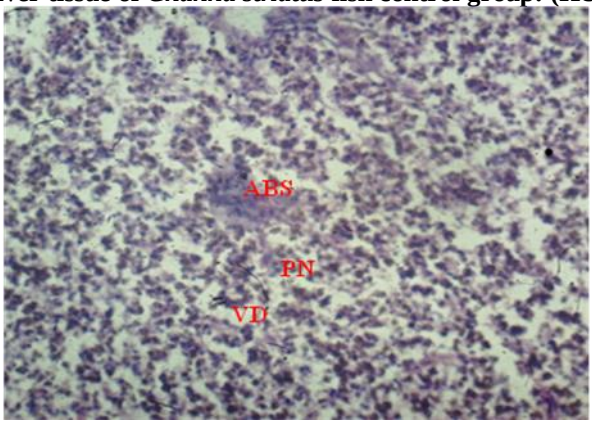

Fig-7: Liver tissue of *Channa striatus* fish exposed to 15 days sub lethal concentration propargite 0.034ppm (H&E 40X).

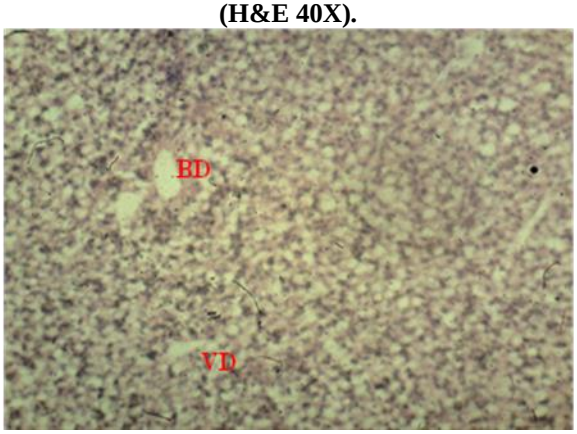

Fig-8: Liver tissue of *Channa striatus* fish exposed to 30 days sub lethal concentration propargite 0.034ppm (H&E 40X).

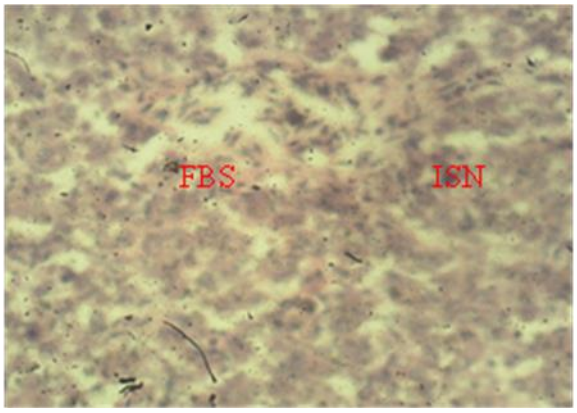

Fig-9: Liver tissue of *Channa striatus* fish exposed to 15 days sub lethal concentration propargite 0.102ppm (H&E 40X).

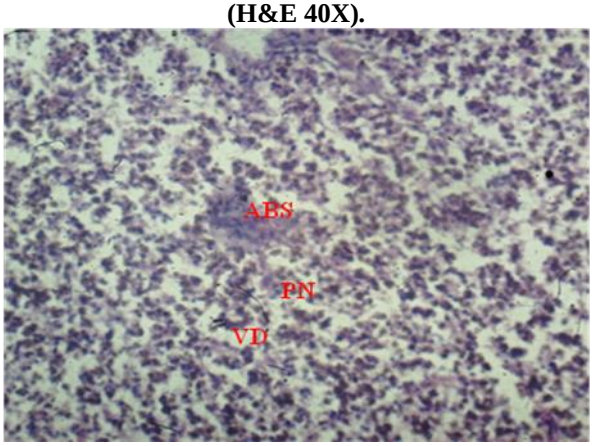

Fig-10: Liver tissue of *Channa striatus* fish exposed to 30 days sub lethal concentration propargite 0.102ppm (H&E 40X).

DISCUSSION:

Pesticides are used extensively in agriculture, and their residues have affected the environment adversely. The histology changes in certain tissues like gills and liver in the fish exposed to sub- lethal concentration of propargite were studied. The present study on gill indicates, primary and secondary gill lamellae, degeneration of pillar cells hepatocytes, epithelium hyperplasia and fusion, sinusoid with blood congestion, necrosis, swelling of the epithelium and excessive mucus secretion in the test animal. This also agreed with the finding. Gill damage due to lindane exposure was observed in the present study. [28] Reported that increased coughing in the common carp following exposure to industrial effluents. Observed such changes in Heteropneusteus fossilis exposed to the pesticide Malathion. Epithelial hyperplasia and curling and fusion of the secondary lamellae were noticed in C. mrigala after exposure to monocrotophos [29]. Gambusia affinis after 30 days of exposure to deltamethrin [30].

Hypotoxic conditions arise primarily due to damage to the gills of fish exposed to pesticides [29] Found that gill is one of the most important organs directly in contact with pollutants and any kind of damage to the gill tissue of fish leads to disorder in the gas exchange process and also the decrease of ion regulation efficiency via this organ [31-32]. Rao *et al.*, (2005) supported that degenerative changes in gill, such as intraepithelial edema in the secondary lamellae, thick coating of mucus covering the entire gill filaments and lamellae, erosion of secondary lamellae, thickening of lamellae, inflammation of epithelial cells, breakages in primary lamellae, degeneration of secondary lamellae, necrosis, rupture of epithelium were noticed during exposure of sub-lethal concentrations of monocrotophos. Uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, epithelial lifting, dilation of the blood sinuses of the secondary lamellae, lamellar aneurism, hemorrhages in the gill of fish exposed to profenofos [33].

Various histopathological studies indicated that gills are the first target of fishes on exposure to pesticides and are prime indicators to show water quality [34]. Gills besides absorbing oxygen perform many important functions such as regulation of ions, acid

base balance and elimination of nitrogenous wastes from body. So, the ecological toxicants affect these vital organs severely and indirectly impose significant effects on health of fish [35]. Showed that deterioration of secondary gill lamellae, necrosis of respiratory epithelium and devastation of primary gill lamellae is also evident after exposure of *Rarbora daniconius* to endosulfan pesticide [36].

Different pesticides showed similar results in different fishes. For instance *C. punctata* exposed to pendimethalin showed cellular hypertrophy with loss in the epithelial layer, fusion of secondary lamellae, cellular degeneration, and necrosis of gill epithelial tissues, epithelial lifting and blood congestion in the vascular axis of primary filaments [37]. Exposure duration was observed, suggesting that a higher dose of the test insecticide probably could activate necrotic processes in the epithelial cells. Overall, all the tested concentrations of Propargite induced the processes, which were mostly associated with epithelial proliferation than degenerative processes.

The present study under sub-lethal concentration of propargite appeared to be more pronounced than liver and includes degenerated haemtopoetic tissue with hemolysis , dilated sinusoids, Ruptured hepatocytes , vacuolization , Ruptured central vein , Congestion , Necrosis , degeneration , edema of hepatocytes , Inflammation . This is similar with the result of histological alterations related to pesticide toxicity in the liver of fish have shown that the substances cause severe damage to the liver cells [38]. Reported that liver is an important organ of detoxification and biotransformation process and due to these reasons the hepatic cells are damaged severely. Several works have reported degenerative changes in hepatic tissue subjected to pollution by various pesticides and insecticides [39].

The liver of pesticide treated fish showed dilation of blood sinusoids, vacuolization, disintegration of cell boundaries and necrosis. The liver is an organ that frequently undergoes changes when exposed to insecticide at sub lethal doses. The changes may be attributed to direct toxic effects of pollutants on hepatic cells, since the liver is the site of detoxification of all types of toxic substances [40].

Similar observations were made by [41]. This showed degeneration and disintegration in most cytoplasmic contents, necrosis along with pyknosis and rupture of hepatocytes on exposure of pentachlorophenol (PCP) to H.fossilis for 21 days. Pyknotic nuclei in liver of malathion treated *H.fossilis* were also observed by [42]. Degenerative changes like vacuolation, karyolysis, severe necrosis, degeneration in central vein with congestion were due to pesticide intoxication [43]. At lethal concentration severe damages were inevitable due to insecticide intoxication. Liver eliminates toxicants through metabolism [44].The liver becomes hyperactive to metabolize and eliminate toxicant. Due to hyperactivity and accumulation of toxicant hepatocytes become larger in size to overcome the metabolic stress which might be the reason for loss of cell membrane and hypertrophy. The severe necrosis might be due to inability of fish to regenerate new liver cells [45]. The lipid infiltration may be due to excessive use of lipids to cope up high energy demands under stress condition.

The liver shows severe alterations in cellular architecture [46] reported on the effect of pesticide on the *Ctenopharyngodon idellus* liver and found degenerative of hepatocytes formation of vacuoles, rupture in blood vessels, necrosis and disappearance of the hepatocytes wall and disposition of the hepatic cords. Hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocytes; focal coagulative necrosis disorganized hepatic canaliculi in *Labeo rohita* exposed to Cypermethrin[47]. Reported that have found rapid and continued destruction of erythrocytes with increased haemolysis and damage of the iron metabolism [48].

The common liver abnormalities loss of parenchymal architecture, fatty degeneration, vacuolar degeneration, atrophy and necrosis of hepatic and pancreatic cells with leucocytic infiltration. The recorded results in the present study were similar to those observed by [49]. Recorded pyknotic nucleus, protein precipitation, pancreatic acini appeared with the loss normal structure and necrosis of the hepatic and pancreatic tissue in freshwater [52-53] fish (*Catla catla*) and (*Oreochromis mossambicus*) treated with chlorpyrifos. Necrosis is probably due to the involvement of liver cells in the metabolic transformation of the insecticide, causing functional and structural changes to the cells [50] reported that chlorpyrifos- ethyl degenerative changes like sinusoidal spaces, massive infiltration of sinusoidal spaces and central vein, pyknotic nuclei, necrosis and coagulation in fish *C. gariepinus.* Liver metabolizes toxins, detoxify them and excrete out through the body. Due to the accumulation, metabolism and detoxification of toxicant the liver cells might be lost their structure and properties even at low concentration. The vacuolization in hepatocytes might indicate imbalance between synthesis of biochemical substances and their release.

CONCLUSION:

The finding of the present study indicates that the exposure sub- lethal concentrations of propargite caused histology changes destructive effect in the gill and liver tissues of *Channa striatus*. Changes in the architecture of gill under propargite stress would alter diffusing capacity of gill with consequent hypoxic/anoxic conditions and thus respiration may become problematic task for the fish. These histological lesions affected the gills by disrupting their functions. Liver is an important organ of detoxification and biotransformation process and due to the hepatic cells, degenerative changes are damaged severely. Gill and Liver tissue changes, such as may result in severe functional problems, ultimately leading to the death of fish. Therefore, controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic pesticides.

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