"An investigation on biochemical and histopathological effects of Rogor in freshwater catfish *Clarias magur* (Hamilton, 1822)"

A teacher-led student research project submitted to the Research cell, Handique Girls' College, Guwahati

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Introduction

Pesticides are mainly used worldwide to control or to manage agricultural pest. According to the United States Environmental Protection Agency (EPA), a pesticide is any substance that is used to destroy, repel, control, or prevent plants and animals considered to be "pests". Pesticides can include fungicides (used to prevent mildew and mold), herbicides (used to destroy weeds), and insecticides (used to repel or kill various insects). Simply, pesticides are specifically designed to be toxic or poisonous to pests.

The impact of pesticides consists of the effects of pesticides on non-target species. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields. Runoff can carry pesticides into aquatic environments while wind can carry them to other fields, grazing areas, human settlements and undeveloped areas, potentially affecting other species. Other problems emerge from poor production, transport and storage practices. Over time, repeated application increases pest resistance, while its effects on other species can facilitate the pest's resurgence.

There are concerns that pesticides used to control pests on food crops are dangerous to people who consume those foods. These concerns are one reason for the organic food movement. Many food crops, including fruits and vegetables, contain pesticide residues after being washed or peeled. Chemicals that are no longer used but that are resistant to breakdown for long periods may remain in soil and water and thus in food. The United Nations Codex Alimentarius Commission (1979) has recommended international standards for maximum residue limits (MRLs), for individual pesticides in food.

Acute health problems may occur in workers that handle pesticides, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems. In China, an estimated half million people are poisoned by pesticides each year, 500 of whom die. Pyrethrins, insecticides commonly used in common bug killers, can cause a potentially deadly condition if breathed in. People can be exposed to pesticides by a number of different routes including: occupation, in the home, at school and in their food.

Widespread use of pesticides in agriculture is a serious problem due to their long-term environmental damage. Some pesticides can stick around for years, posing a very real threat to

the ecological system and hence human health. Excessive and careless use of pesticides can contaminate water sources and soil, make fruits and vegetables less nutritious, and reduce biodiversity. Pesticides enters into our bodies through our diets when we eat veggies and fruits with high amounts of pesticide residue. Some pesticides have also been linked to the dramatic reduction in the number of bees across the world, posing a huge threat to agriculture and food security, given that bees pollinate more than 70% of all crops.

Studies by the UK government (Bingham, 2007) showed that pesticide concentrations exceeded those allowable for drinking water in some samples of river water and groundwater. Pesticide impacts on aquatic systems are often studied using a hydrology transport model to study movement and fate of chemicals in rivers and streams. There are four major routes through which pesticides reach the water: it may drift outside of the intended area when it is sprayed, it may percolate, or leach through the soil, it may be carried to the water as runoff, or it may be spilled, for example accidentally or through neglect. They may also be carried to water by eroding soil (Papendick et al., 1986). Factors that affect a pesticide's ability to contaminate water include its water solubility, the distance from an application site to a body of water, weather, soil type, presence of a growing crop, and the method used to apply the chemical.

The importance of fish as source of high quality balanced and easily digestible protein, vitamins and polysaturated fatty acids is well understood (Shamsan and Ansari, 2010).Fishes are the valuable sources of high grade protein and other organic products. They are the most important source of animal protein and have been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Andrew, 2001). But the poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long term effect may result in the incidence of poisoning of fish and other aquatic life forms. Pesticide surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream. Many insecticides have shown to effect the growth and reproduction in fishes with evidence of tissue damage. The severity of damage depends on the toxic potentiality of a particular compound or insecticide accumulated in the tissues. Environmental fluctuations create hormonal and biochemical alterations that determines the quality and health status of fish. Fishes accumulate heavy metals in their body at dangerous level. The food chain in the ecosystem passes the energy as well as toxic metals to the higher levels. In humans, heavy metal like lead causes learning dysfunction, mental retardation

and loss of coordination

Among different classes of pesticides, organophosphates are most commonly used, because of their high insecticidal property. Rogor (Dimethoate) is one of the organophosphate extensively used in agriculture practice. Rogor is highly soluble in water for which it can easily enter into the nearby water sources and affect aquatic organisms. On the other hand, *Clarias* is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Among which *Clarias magur* is one of the commonly reared fish in Asian countries and is an edible fish found in ponds. The fish is popular for its tasty flesh, rapid growth and high market price. Based on these view, we have made an attempt to investigate the influence of organophosphate pesticide Rogor on biochemical changes in liver and muscles, and histopathological alteration in the liver of freshwater catfish *Clarias magur.*

Objectives

At first we planned to visit a well-established fishery Institute to gain knowledge on handling and maintenance of experimental fishes, and to achieve some ideas to carry out the experimental parameters.

Then we have planned to carry out an investigation on the effects of the insecticide rogor on freshwater catfish *Clarias magur* after exposing them for 7 days, with the following objectives:

1. To estimate the total protein in liver and muscle
2. To estimate the total glycogen in liver and muscle
3. To estimate the total lipid content in liver and muscle
4. To estimate blood glucose
5. To estimate blood cholesterol
6. To investigate histopathological alteration in liver
7. To determine statistical significance of the results

Review of literature

Fishes are very sensitive to a wide variety of toxicants in water. Toxicity studies have long played an important role in man's efforts to monitor and modify the effects of his activities on the biota (Bhandare et al., 2011).Various experiments have been conducted since an earlier time to test the genotoxic effects, biochemical alterations, histopathological effects and its bioaccumulation in different species of freshwater fishes(George et al, 2017; Nwani et al, 2010; Soundararajan and Veeraiyan, 2014). Catfishes are attracting attention of the pisciculturists owing to their high production potential from paddy fields and stagnant shallow ponds (Bagchi et al; 1990). Begum and Vijayaraghavan (1999) observed a gradual decrease in muscle glycogen and an increase in lactate contents when treated with rogor in Clarius. In another study, Prakriti et al (2016) observed an alteration in free amino acids and protein content in *Clarias batrachus* after treated with Endocel and Rogor. Therefore, a thorough research study on the effects of rogor on *clarias magur* should begin with a thorough historical overview of the context of various experiments.

Fish liver is an excellent organ for the study of toxicity effects, due to its role in the animal metabolism, which include the production of protein, the oxidation, conjugation, methylation, inactivation or detoxification of substances, or rather and the excretion of pollutants (Fanta et al., 2003). Protein is the most primary biochemical ingredient present in large quantity in the fish body. Ferrari et al.(2007) reported damaged muscle fibre with discontinued `H' and I band in fish, *Hplias malabaricus* exposed to contamination of mercury. Liver is an important organ involved in metabolic processes and in the detoxification and xenobiotics, which was analyzed by Yang and Chen in 2003.The chemical composition of protein and lipids is traditionally used as an indicator of the nutritional value as well as the physiological condition of fish and its habitat (Moghaddam, 2007;Aberoumad and Pourshafi, 2010).

Ansari and Kumar (1988) reported that the exposure of zebra fish to diazinon for 168 hrs has significantly reduced DNA, RNA, and the total protein in the liver, but significantly increased the amino acid content in a dose and time-dependent response. Lipids play an important role in virtually all aspects of biological processes in the body. Disturbances of its level in tissues and serum are usually associated with many abnormalities, including gallstone

formation, atherosclerosis, and coronary artery disease (Moss et al., 1987). Ibrahim and El-Gamal (2003) observed a significant decrease in plasma total cholesterol level with the 1/8 LD_{50} dose level of diazinon in male albino rats.

Liver is the primary organ for detoxification (Hulterer et.al., 1969) and hence it is expected that toxicant could reach there for detoxification and disposal. This results in structural changes in the liver, the arrangement of hepatic cords leading to the alteration of liver metabolism and its biochemical content. The pollutants act as one kind of stress and organism respond by developing necessary potential occurring in body give first indication of stress. During stress organism needs sufficient energy which is supplied from reserve food material i.e. protein, glycogen, cholesterol etc.

A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemical. Ganeshwade (2011) observed a progressive decrease in the protein content of the liver of fresh water fish *Puntius ticto* with increase in concentration of two sublethal doses of Dimethoate. Thus the toxicity of dimethoate showed a direct correlation with the concentration. Similar observation has been observed by Singh and Bhati (1994).Binukumari and Vasanthi (2013) observed a decreased level of liver and muscle protein in *Labeo rohita* when exposed to an insecticide Encounter. Verma et al.(2015) observed significantly decrease total protein in blood serum in the fishes *Clarias batrachus* exposed to pesticides Endocel and Rogor. Normal levels of protein in different tissues and serum is essential for the metabolic harmony of the organism. Protein profile of cells and tissues gives an indication of physiological status of an animal and there exists a dynamic equilibrium between the synthetic and degradation pathways. Protein level may be altered if there is interference with either the synthetic or degradative pathway. Singh et al. (2015) found a significant decrease in muscle and liver protein in *Cyprinus carpio* at both short and long term exposure of dimethoate.

The blood glucose level can be an indicator of biological stress caused by pollutants such as pesticides and metals (Silbergeld, 1974). Oluah and Chineke (2014) observed significantly decrease liver glycogen, liver protein and serum glucose concentrations with increasing λ-cyhalothrin test concentration in the african catfish *Clarias gariepinus*. In the same experiment they also observed significantly increase serum cholesterol level. A reduction in hepatic glycogen indicating impairment in the carbohydrate metabolism was reported in *Anabas testudineus* treated with acute lethal and sublethal concentrations of furadan (Bhakthavalsalam

and Reddy, 1982). A reduction in glycogen content of liver and muscle of *Channa punctatus* exposed to endosulfan was reported by Sastry and Siddiqui (1983). Exposure to sublethal and lethal doses of the pesticide thiodon elicited a severe hypoxia in the fish *Sarotherodon mossambicus* resulting from the utilization of stored glycogen by way of anaerobic glycolysis to meet the demand of energy during pesticide stress as evidenced by a fall in the glycogen content of the liver, muscle and heart (Vasanthy and Ramaswami, 1987). A decrease in the liver glycogen in the fish *Anabas testudineus* exposed to sublethal concentration of pulp and paper mill effluent was reported by Vijayaram et al. (1991). A study conducted by Ferrando and Andreu-Moliner (1991) on the effect of exposure of the European eel, *Anguilla anguilla* to the sublethal doses of insecticide, lindane showed a decrease in muscle glycogen level. Gimeno et al. (1995) observed a decrease in muscle glycogen and an increase in blood glucose in the European eel, *Anguilla anguilla* exposed to sublethal concentrations of endosulfan.

Liver glycogen content decreased progressively during pesticide exposure period. This may be due to toxic stress. During stress an organism needs sufficient energy which is supplied from reserved glycogen. Glycogen is stored in the organism mainly in the liver and muscles in the form of carbohydrate. It may provide a reserve food for acute demands recurring as a result of transient stress (Love 1980). A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicants through glycolysis or hexose monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Radhaiah et.al., (1987) observed decreased carbohydrate content in heptachlor intoxicated fish *Tilapia mossambica* and stated that this may be due to the rapid utilization of carbohydrates by the tissue possibly to overcome the pesticides induced stress. James and Sampath (1995) observed decreased liver glycogen in the *Heteropneustes fossilis* (Bloch) under mixtures of copper and ammonia; and reported glycogenolysis releasing glucose in to the circulatory system to meet increased energy demand during stress conditions. Susan et. al. (1999) observed drastic decrease in glycogen content in liver of *Catla catla* under fenvalerate toxicity stress. Rawat et.al. (2002) have reported continuous decrease in quantity of glycogen in the liver of *Heteropneustes fossilis* exposed to endosulfan. Decrease in the glycogen level in liver suggests the possibility of glycogenolysis. A study indicating such depletion in fish models (Mishra and Srivastava 1984) during organophosphorus toxicity offers an excellent support to the decreasing levels of glycogen in the

present study. Khan et. al., (1992) observed significant decrease of cholesterol in liver of Cd treated fish *Garra mullya* and stated in may be due to general damage in liver. Shakoori et. al., (1996) studied effect of sublethal dose of fenvalevate on the liver of fish *Ctenopharyngodon idella* and observed decreased level of cholesterol. Virk and Sharma (1999) studied biochemical changes induced by nickel and chromium in the liver of *Cyprinus carpio* and observed significant decline in the cholesterol level of liver and stated this may be due to toxicity stress which suppresses the activity of a number of enzymes responsible for lipid transformation ultimately causing disturbance in lipid metabolism and leads to decrease in values of cholesterol.

The liver in animals is greatly affected by the pesticidal contaminants present in their environment. Sastry and Sharma (1978) reported that treatment with endrin produced acute pathological changes in the liver of *Channa punctatus*. They observed hypertrophy of hepatocytes within 8 hours after the injection of the pesticide. The centrolobular portion of the liver was necrosed and the nucleus showed an increase in size and in some liver cells, cell membranes were ruptured. In another study, Saxena and Sarin (1980) reported that a single injection of phorate (2.5 mg/kg) to the desert gerbil, *Meriones hurrianae* showed mild to severe pathological changes in liver. They also observed cloudy swelling of hepatocytes, cytoplasmolysis and cytoplasmic vacuolization. The nuclei of hepatocytes showed karyolysis and karyorrhexis. Singh et al. (1981) studied the alterations in the ultrastructure of hepatocytes by electron microscopy in rats fed with photomirex or mirex. There was selective ultrastructural damage to the mitochondria of hepatocytes. The parenchymal cells had a proliferation of smooth surfaced ER with a concomitant depletion of glycogen, a reduction of rough ER, an abnormal accumulation of lipid droplets and pleomorphic nuclei at 5 ppm photomirex level. Virk et al. (1987) observed that the polygonal structure of the hepatic cells has been lost and the whole structure formed a compact mass of cells in the liver of the fish *Mystus tengara* when treated with 0.0015 ppm endrin and 5 ppm carbaryl for 28 days. At certain places necrosis and atrophy also occurred; vacuolization and splitting appeared at various places. *Tilapia mossambica* when exposed to sublethal concentration of 0.009 mg /L of fenvalerate for 10 and 20 days showed histopathological lesions such as vacuolated hepatocytes, necrosis, pushing of nuclei and degeneration of cytoplasm. The effects were more pronounced for the longer duration of exposure (Radhaiah and Rao, 1992). Srivastava and Srivastava (1998) reported vacuolar degeneration of the cytoplasm, localized necrosis and hypertrophy of hepatic cells in the cat fish

Heteropneustes fossilis treated with the pesticide chlordecon. Swelling of hepatocytes with diffuse necrosis and marked swelling of blood vessels were observed in the liver tissue of the Indian major carp, *Labeo rohita*when exposed to sublethal concentration of hexachlorocyclohexane (Das and Mukherjee, 2000a). The organophosphate insecticide Dipterex 500® (Trichlorfon) at a concentration of 0.2µl/L for 48 hours caused alterations in hepatic tissue including lateral migration of nuclei, variation in the diameter and density of nuclei, pyknosis and necrosis in the fish *Prochilodus lineatus* (Rodrigues et al., 2001). Liver tissue revealed atrophy, appearance of blood streaks among hepatocytes and changes in the haemopoietic tissue in *Cirrhinus mrigala* exposed to fenvalerate (Susan and Tilak, 2003). Sarkar et al. (2005) studied alterations in the liver histology of *Labeo rohita* (Hamilton) after exposure to different concentrations of carbofuran and cypermethrin. Major damages caused by carbofuran were diffused necrosis, cordal disarrangement and individualization of hepatocytes; and significant changes induced by cypermethrin were hyperplasia, disintegration of hepatic mass and focal coagulative necrosis. In both cases damages were dose dependent. On exposure to sublethal and lethal concentrations of technical grade as well as 20% E.C. of chlorpyrifos for 8 days the freshwater fish *Catla catla* showed marked pathological changes in the liver viz., degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis, and disappearance of hepatic cell wall and disposition of hepatic cords (Tilak et al., 2005a). Tilak et al. (2005b) also observed similar kind of pathological symptoms in the fish *Cirrhinus mrigala* exposed to the same pesticide.

Materials and methods

Collection and maintenance of experimental fishes: The current works had been carried out from June 2019 to March 2020. We collected the freshwater catfish *Clarias magur* (fig.1.) from local market of Guwahati. Earthen pots (Fig.2.) covered with mosquito net were used to culture the experimental fishes (3 pots were used, each with capacity of 20 liters). Fishes were treated with 0.1% $KMnO_4$ for five minutes to get rid of any dermal infection. The fish weighing 100±10 gms were selected for the works. Fishes were maintained at temperature of 27±1°C in our laboratory conditions for acclimatization. They were allowed to acclimatize for 15 days. During this period, the fishes were fed twice a day with the commercial fish food, mosquito larvae and chironomous larvae to avoid their starvation. The water used was tap water containing pH 7.2±0.3 and dissolved oxygen 7.9mg/L±0.2. Twenty-four hours before starting the experiment, the food was stopped to clear off the alimentary canal.

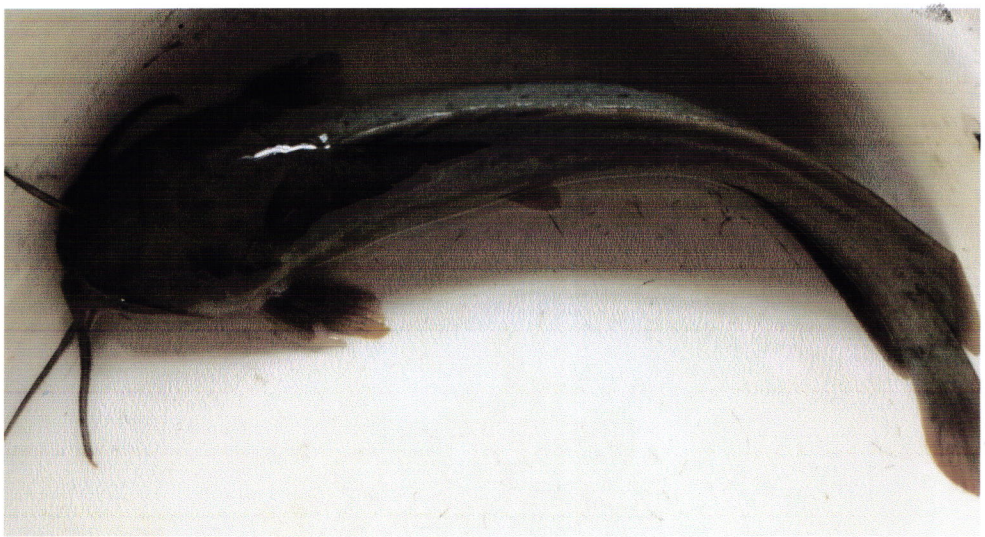

Fig. 1. Experimental fish (*Clarias magur*) collected from local market

Fig.2.Earthen pot used to culture experimental fishes (3 pots were used, each with capacity of 20 liters)

Fig.3.Rogor collected from market

Chemical used in the experiment:

The organophosphorus pesticide Rogor (dimethoate 300g/liter or 30% EC), is collected from local market (Fig.3.). Dimethoate [IUPAC Name- O, Odimethyl S-(N-methylcarbamoylmethyl) phosphoro-dithioate] is a systemic insecticide widely used for controlling insect pests of fruits, vegetables and crop plants. It was first patented and introduced in the 1950's by American Cyanamid.

Dimethoate is non-photodegradable, undergoes very slow hydrolysis and shows moderate persistence in water. Like other organophosphates dimethoate irreversibly inhibits acetyl cholinesterase enzyme which is essential for Central Nervous System function and works primarily as nerve poison. It is available in aerosol spray; dust, emulsifiable concentrate, and ultra-low volume concentrate formulations.

It acts by contact, ingestion, inhalation and dermal absorption. As with all organophosphates, dimethoate is readily absorbed & distributed. The effects are usually includes bloody or runny nose, coughing, chest discomfort etc. Severe poisoning will affect the central nervous system

Determination of sub-lethal concentration:

The LC_{50} value (lethal concentration) of Rogor for *clarias magur* is 65mg/liter for 96 hrs. (Begum & Vijayaraghavan, 1999). Based on this value, in our present study, we used two sub-lethal concentration of Rogor - $1/5^{th}$ (i.e; 13 mg/L) and $1/10^{th}$ (i.e; 6.5mg/L) of LC_{50} value.

Treatments and applied doses of the chemical: In the experiment, fishes were randomly divided into 3 experimental groups, of 6 fishes each and treated as follows:

- Group1 treated as control
- Group 2 treated with sub-lethal concentration of Rogor 6.5 mg/L
- Group 3 treated with sub-lethal concentration of Rogor 13mg/L

Treatment was carried out for seven consecutive days. After 24 hours of the last treatment, fish blood was obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant. Then they were sacrificed for other parameters. Our departmental teachers and elder students (MSc) cooperate us to collect blood from the fishes (Fig. 4., 5.)

Fig. 4. At the time of blood collection from experimental fishes

Fig.5.At the time of biochemical estimation

Separation of required samples for investigation: Treatments were carried out in two different dose levels for 7 consecutive days. After 24 hours of the last exposure period, blood samples were obtained by severance of caudal peduncle. Fishes were then sacrificed to isolate liver and muscle tissues for other biochemical and histological parameters.

Methods for biochemical and histological parameters:

For estimation of total protein, glycogen and lipids from liver and muscle, 1gm of each tissue was transferred into the homogenizer containing 10%TCA (Tri chloro acetic acid); and then processed to prepare tissue extract for different biomolecule estimation.

From tissue extract, protein content was estimated by Folin phenol reagent method (Lowry et.al.,1951); Glycogen content was analyzed by using Anthrone reagent method (Zwaan and Zandee, 1972). Gravimetric method was used to estimate total lipid contents.

To estimate total Cholesterol in blood, standard kit (ROBOniK) was used; and for blood glucose, Glucometer (Dr. Morepen, GlucoOne) was used.

For histological study routine (hematoxylin and eosin stain) staining procedure was used.

Statistical Analysis: For the experimental parameters, the data obtained were statistically analyzed by using mean ± S.D (Standard Deviation). One-way ANOVA test was used to derive significant difference between means through SPSS.

A visit report of the College of Fisheries

Before starting of the experiments, we went to the College of Fisheries, Assam Agricultural University, Raha, Nagaon on 14th June, 2019 (fig. 6., 7.). From that Institute we have learned lots of thing regarding how to handle experimental fishes, how to carry out different experiment etc. They thoroughly trained us the histological procedure to be carried out with fishes. They told us about different types of fish food (fig. 8.). Out of these fish foods, we used mosquito larvae and chironomous larvae to feed the experimental fishes. Dr. Kaustabh Bhagawati, Dept of Aquaculture demonstrated us preparation of fish foods and fish breeding techniques (fig. 9., 10., 11). He also assisted us to visit the different labs of the institute. Mr. Raktim Sharma, M.Sc student, dept of Fish Pathology demonstrated the techniques used to study the effects of pesticide on fish (fig.12.). Dr. Inam Akhtar Hussain, Dept. of Fish Processing, demonstrated us the use of HPLC technique to estimate fish protein (fig. 13.). Dr. Binod Kalita, Dept of Aquaculture demonstrated us the histological techniques (fig. 14.) for different types of tissues.

Some of the photographs of that Institute at the time of our visits are included here.

Fig. 6. Campus of the College of Fisheries, Assam Agricultural University, Raha, Nagaon.

Fig. 7.Our team at College of Fisheries, Assam Agricultural University, Raha, Nagaon.

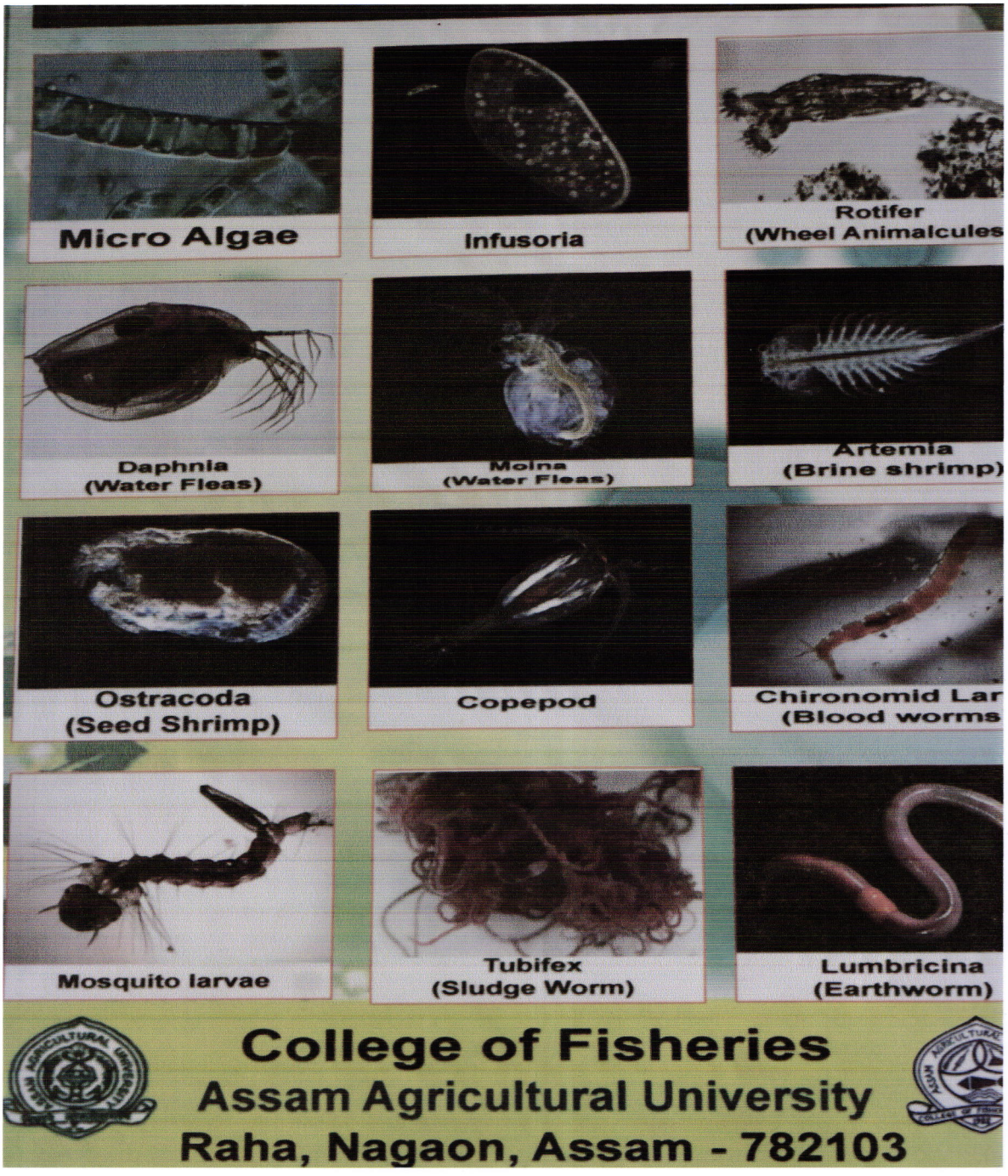

Fig.8. Different types of fish food. Out of these fish foods, we used mosquito larvae and chironomous larvae to feed the experimental fishes.

Fig.9. Fish food production Lab

Fig.10. Fish breeding Lab

Fig.11. Dr. Kaustabh Bhagawati, Dept of Aquaculture demonstrated us preparation of fish foods. He also assisted us to visit the different labs of the institute.

Fig. 12.Mr. Raktim Sharma, M Sc student, dept of Fish Pathology demonstrated the techniques used to study the effects of pesticide on fish.

Fig.13.Dr. Inam Akhtar Hussain, Dept. of Fish Processing, demonstrated us the use of HPLC technique to estimate fish protein.

Fig.14. Dr. Binod Kalita, Dept of Aquaculture demonstrated us the histological technique

Results

In our experiment, we observed decrease protein (table 1 and fig. 15.) and glycogen (table 2 and fig. 16.) content in both liver and muscle. The decrease amount of protein and glycogen was dose dependent. The amount of protein and glycogen was decreases with the increasing concentration of the rogor. The results were significant at 0.1 ($P<0.1$). Total lipid content (table 3 and fig. 17) in liver and muscle also decreases in rogor treated fishes. The decrease amount was dose dependent and the results were significant at 0.05 ($P<0.05$). Blood glucose and total cholesterol content (table 4 and fig. 18.) in rogor treated fishes were also decreases with the increasing concentration of the rogor. The results were significant at 0.1($P<0.1$).

Table 1: Total protein in liver and muscle in control and rogor treated *C. magur.*

Experimental groups	Liver protein (mg/gm)	Muscle protein (mg/gm)
Control	120.18±6.98	128.89±3.72
6.5mg/L	98.35±3.87	107.12±5.96
13mg/L	77.64±5.21	92.19±6.00

Each value is mean ±SD of six observations (+ indicates increase over control, -indicates decrease over control, results are significant at 0.1 ($P<0.1$).

Table 2: Total glycogen in liver and muscle in control and rogor treated *C. magur.*

Experimental groups	Liver glycogen (mg/gm)	Muscle glycogen (mg/gm)
Control	38.01 ± 3.61	31.22 ± 3.30
6.5mg/L	24.27 ± 2.42	18.99 ± 2.35
13mg/L	10.87 ± 1.56	10.24 ± 2.22

Each value is mean ±SD of six observations (+ indicates increase over control, -indicates decrease over control, results are significant at 0.1 ($P<0.1$).

Table 3: Total lipid in liver and muscle in control and rogor treated *C. magur.*

Experimental groups	Liver lipid (mg/gm)	Muscle lipid (mg/gm)
Control	0.073±0.005	0.085±0.008
6.5mg/L	0.067±0.008	0.079±0.006
13mg/L	0.055±0.005	0.07±0.006

Each value is mean ±SD of six observations (+ indicates increase over control, -indicates decrease over control, results are significant at 0.05 ($P<0.05$).

Table 4: Blood glucose and Total cholesterol in blood in control and rogor treated *C. magur*

Experimental groups	Blood glucose (mg/dl)	Total cholesterol in blood (mg/dl)
Control	108.67±6.87	187.20±3.44
6.5mg/L	92.83±3.40	168.51±3.32
13mg/L	78.67±2.88	157.61±4.02

Each value is mean ±SD of six observations (+ indicates increase over control, -indicates decrease over control, results are significant at 0.1 ($P<0.1$).

In the present study we also observed some changes in histological structure of the liver of rogor exposed fishes. The liver sections of the fishes from control group showed normal histological structure of central vein, arteries and hepatocytes (fig.19.). Whereas, the liver section of the fishes exposed to both low and high doses (6.5 and 13mg/L respectively) of rogor showed hydropic changes (fig. 20.), infiltration of leukocyte cells in portal vein (fig. 21.) and blood spilling into the liver tissues (fig. 22.). These changes were more pronounced in the liver section of the fishes exposed to high dose (13mg/L) of rogor.

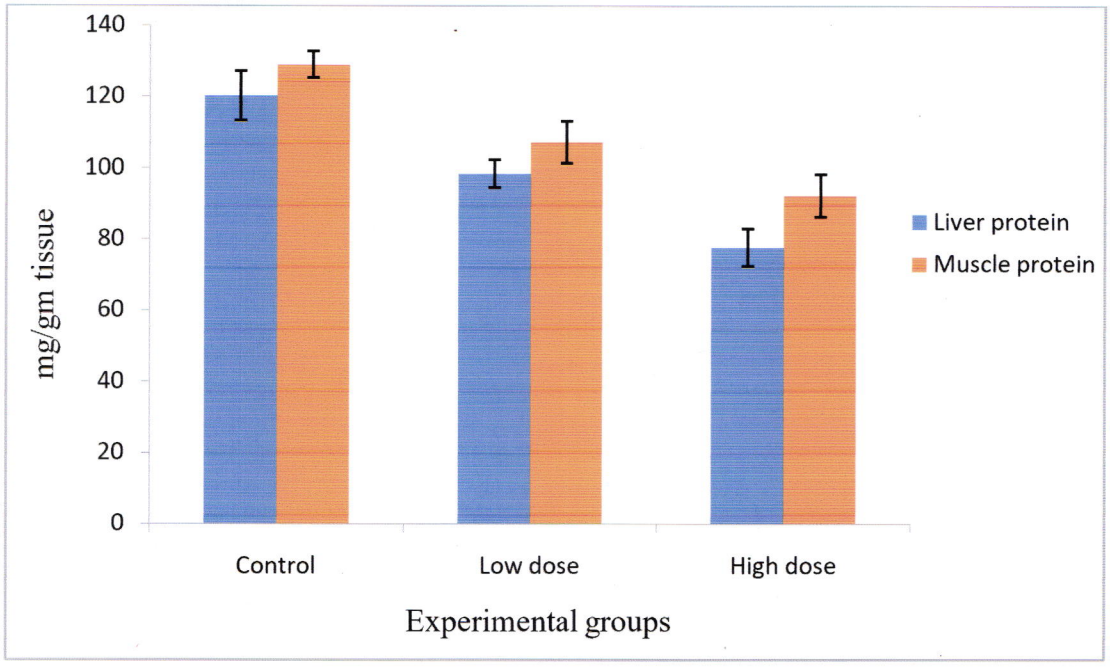

Fig.15. Total protein content in liver and muscle of control and rogor treated *C. magur.* Each value is mean ±SD of six observations and the results are significant at 0.1 (P<0.1).

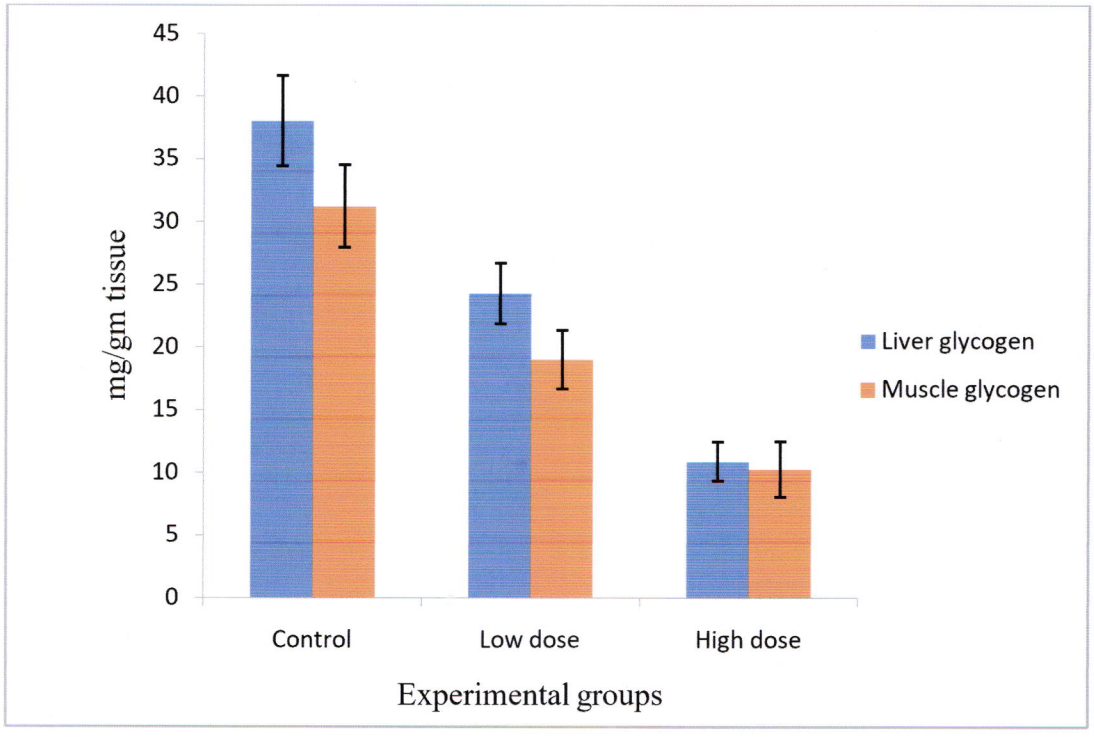

Fig. 16. Total glycogen content in liver and muscle of control and rogor treated *C. magur.* Each value is mean ±SD of six observations and the results are significant at 0.1 (P<0.1).

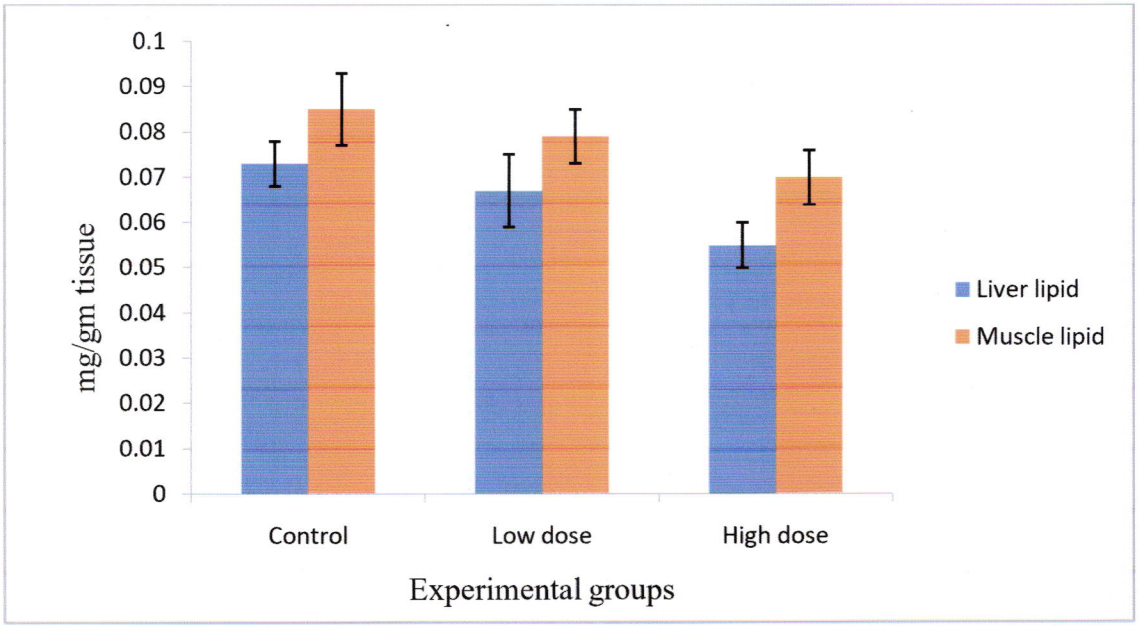

Fig. 17. Total lipid content in liver and muscle of control and rogor treated *C. magur*. Each value is mean ±SD of six observations and the results are significant at0.05 (P<0.05).

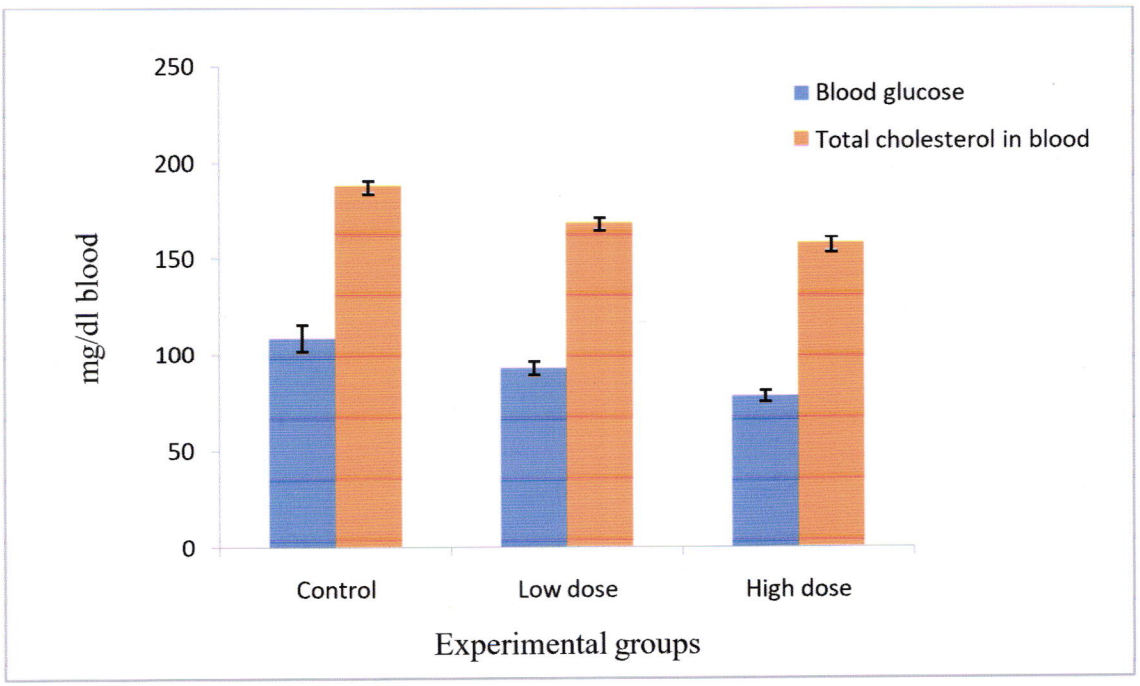

Fig. 18. Blood glucose and Total cholesterol level in blood of control and rogor treated *C. magur* Each value is mean ±SD of six observations and the results are significant at 0.1 (P<0.1).

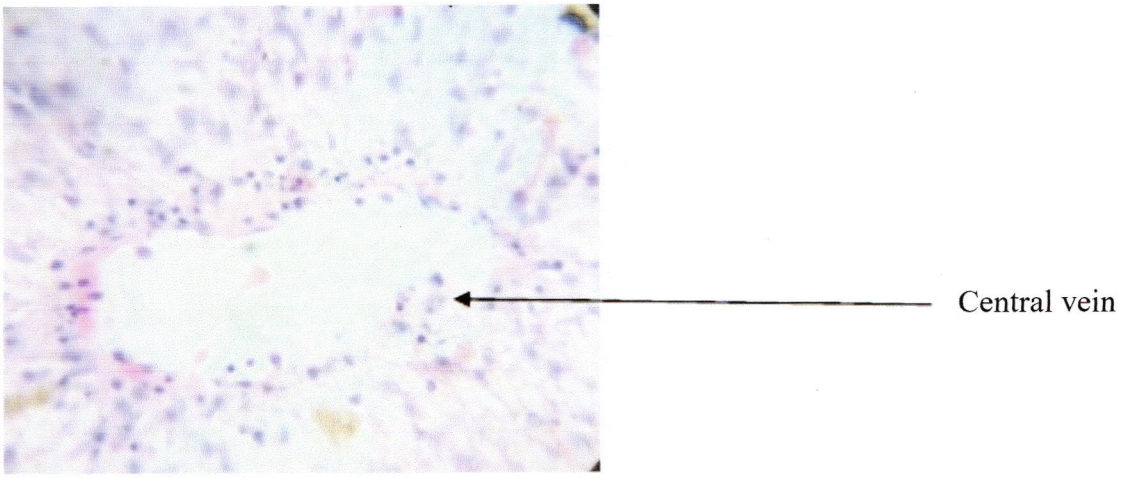

Fig. 19. Liver section of control fishes showing the structure of normal hepatocytes and central vein (H&E, X100).

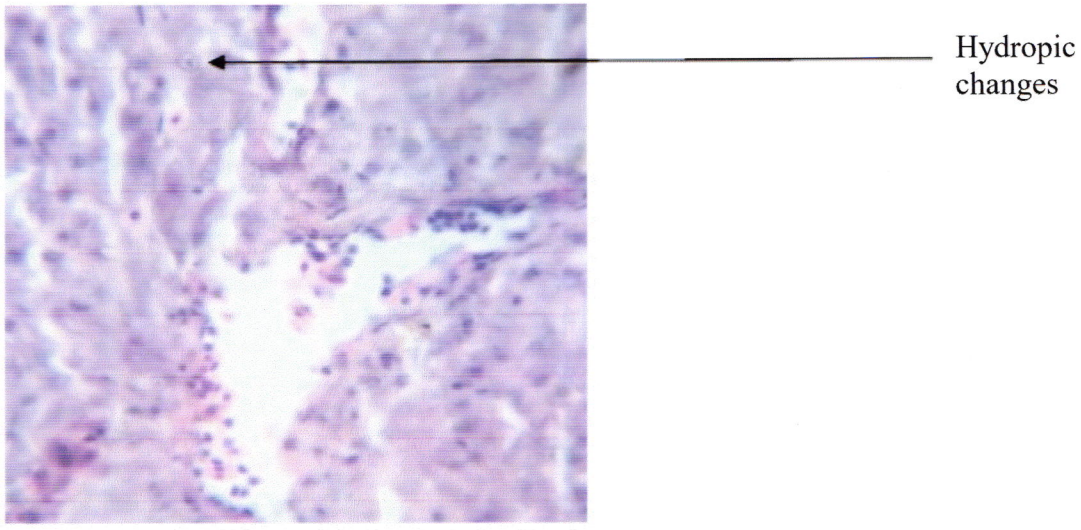

Fig.20.Liver section of rogor treated fishes showing hydropic changes (H&E,X100)

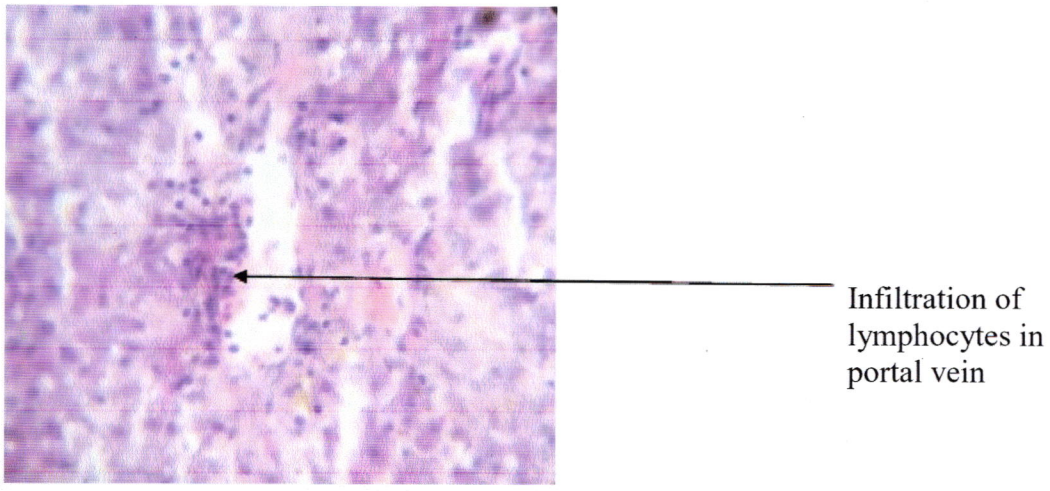

Fig. 21. Liver section of rogor treated fishes showing infiltration of lymphocytes in portal vein (H&E, X 100)

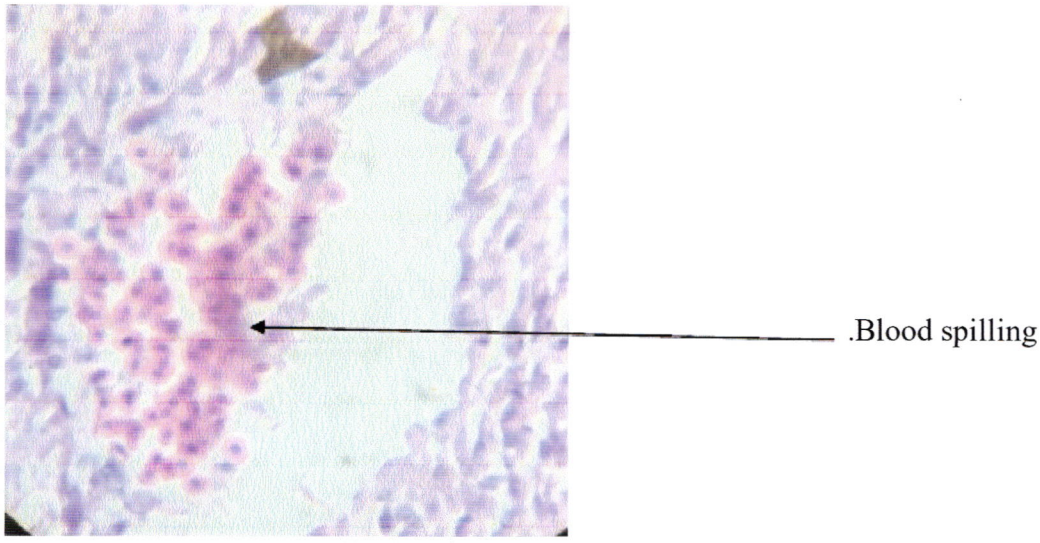

Fig. 22. Liver section of rogor treated fishes showing blood spilling (H&E, X 100)

Discussions

The current study provides an idea to assess health status of fish and the serious ecological consequences of pollutants in freshwater communities. In our present works, we observed decrease protein (table 1 and fig. 15.) content in both liver and muscle of rogor treated fishes. The amount of protein was decreases with the increasing concentration of the rogor and the results were significant at 0.1 ($P<0.1$).The decrease of total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism or this may bedue to liver damage where most protein synthesis usually occurs. These results agreed with that of Sing and Sarma (1998), who reported that carbosulfan treatment in *Clarias batrachus* resulted in drastic decrease in the protein content of gill, brain, muscle, liver, kidney and heart of the fish. Under conditions of stress many organisms will mobilize proteins as an energy source via oxidation of amino acids. The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes as reported by Ravinder and Suryanarayana (1985).Fish liver is an excellent organ for the study of toxicity effects, due to its role in the animal metabolism. Protein is the most primary biochemical ingredient present in large quantity in the fish body. Ferrari et al.(2007) reported damaged muscle fibre with discontinued `H' and I band in fish, *Hplias malabaricus* exposed to mercury contaminated water.

Ganeshwade (2011) reported a progressive decrease in the protein and glycogen content in the liver of *Puntius ticto* with increase in concentration of Dimethoate. The toxicity of dimethoate showed a direct correlation with the concentration and time exposure. Liver is an important organ involved in metabolic processes and in the detoxification and xenobiotics (Yang and Chen in 2003). The chemical composition of protein and lipids is traditionally used as an indicator of the nutritional value as well as the physiological condition of fish and its habitat (Moghaddam, 2007; Aberoumad and Pourshafi, 2010).

We also observed a decrease in glycogen (table 2 and fig. 16.) content in both liver and muscle of rogor treated fishes. The amount of glycogen was decreases with the increasing concentration of the rogor and the results were significant at 0.1 ($P<0.1$). Decrease in liver glycogen content may be due to toxic stress. During stress an organism needs sufficient energy which is supplied from reserved glycogen. Glycogen is stored in the organism mainly in the liver and muscles in the form of carbohydrate. A reduction in glycogen content of liver and muscle of

Channa punctatus exposed to endosulfan was reported by Sastry and Siddiqui (1983). A decrease in the liver glycogen in the fish *Anabas testudineus* exposed to sublethal concentration of pulp and paper mill effluent was reported by Vijayaram et al. (1991). A study conducted by Ferrando and Andreu–Moliner (1991) on the effect of exposure of the European eel, *Anguilla anguilla* to the sublethal doses of lindane showed a decrease in muscle glycogen level.

In our study, we observed a dose dependent decrease in total lipid content (table 3 and fig. 17) in liver and muscle of rogor treated fishes. The results were significant at 0.05 ($P<0.05$). Our results were supported by the findings of Rao et al. (1985), who reported a decrease in total lipid content in the fish *Channa punctatus* after exposure of carbaryl and fenthoate, in both combined and individual application

We observed a dose dependent decrease in blood glucose (table 4 and fig. 18.) level in our experiment. The blood glucose level can be an indicator of biological stress caused by pollutants such as pesticides and metals (Silbergeld, 1974).Oluah and Chineke (2014) observed significantly decrease liver glycogen, liver protein and serum glucose concentrations with increasing λ-cyhalothrin test concentration in the african catfish *Clarias gariepinus*.

In our experiment, we also observed a dose dependent significant decrease in total cholesterol content in blood (table 4 and fig. 18.) of rogor treated fishes. This might be due to the impaired metabolism of liver. Gill et al. (1991) reported a decrease in blood cholesterol in endosulfan treated fishes. Ceron et al. (1996) also reported a decrease in plasma cholesterol and triglyceride contents during 96 hrs treatment with sublethal concentration of diazinon in the fish *Anguilla anguilla*.

In the present study we also observed some changes in histological structure of the liver of rogor exposed fishes. The liver sections of the fishes from control group showed normal histological structure of central vein, arteries and hepatocytes (fig.19.). Whereas, the liver section of the fishes exposed to both low and high doses (6.5 and 13mg/L respectively) of rogor showed hydropic changes (fig. 20.), infiltration of leukocyte cells in portal vein (fig. 21.) and blood spilling into the liver tissues (fig. 22.). These changes were more pronounced in the liver section of the fishes exposed to high dose (13mg/L) of rogor. Kalita and suman (2018) reported similar effects in the liver of *Clarias magur* when treated with sublethal concentration of rogor, 6.5 mg/L for 7 days. The liver in animals is greatly affected by the pesticidal contaminants present in their environment (Mukherjee and Bhatacharya, 1975). Srivastava and Srivastava (1998)

reported damaged liver cells, vacuolar degeneration of the cytoplasm, localized necrosis and hypertrophy of hepatic cells in the cat fish *Heteropneustes fossilis* treated with the pesticide chlordecon. Swelling of hepatocytes with diffuse necrosis and marked swelling of blood vessels were observed in the liver tissue of the Indian major carp, *Labeo rohita* exposed to sublethal concentration of hexachlorocyclohexane (Das and Mukherjee, 2000 a).

Conclusion

Pesticides are often considered as a quick, easy, and inexpensive solution for controlling insect pests in agriculture. The primary benefits of pesticides are the direct gains expected from their use in agriculture. For example, the effect of killing caterpillars feeding on the crop brings the primary benefit of higher yields and better quality of a particular plant. If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre, and destruction of vector-borne diseases, then their debits have resulted in serious health implications to man and environment. Pesticides have contaminated almost every part of our environment. Pesticide residues are found in soil, air, and in surface and ground water across the countries. Pesticide contamination poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms, to insects, plants, fish, birds, mammals etc.

There is now overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment. No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects. The world-wide deaths and chronic diseases due to pesticide poisoning is about 1 million per year. Thus pesticides present widespread risks to human and environmental health. Highly toxic pesticides are still in widespread use internationally and constitute a substantial challenge to human health.

The data on environmental-cum-health risk assessment studies may be regarded as an aid towards a better understanding of the problem associated with the use of pesticides. The importance of education and training of agricultural workers should be taken as a major vehicle to ensure a safe use of pesticides. There is a need to convey the message that prevention of adverse health effects and promotion of health are profitable investments to a sustainable development of economics. The best way to reduce pesticide contamination and its harmful effects on the environment, is to do a safer use of pesticides and as far as possible implementing biological pest control methods. There is thus every reason to develop health education packages based on knowledge, skill and practices and to circulate them within the community in order to minimize human exposure to pesticides.

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