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Blood Performance, Enzymatic Alteration and Recovery study on *Heteropneustes fossilis* (Bloch) Exposed to Pyrethroids

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ABSTRACT: Population explosion and industrial domination in third world countries like India, is the major cause for hazardous damage of ecosystem, especially aquatic ecosystem and aquatic organisms. From agricultural field and house hold wastes containing pesticides released in the waterbodies directly or indirectly is highly responsible for water pollution. The polluted water caries different health hazards to the body of aquatic organisms. The hazards maybe either physiological, biochemical or behavioural, which can directly impact on the health status of aquatic organism. Cat fish like *H. fossilis, C. magur* are considered as a pure source of protein and nutrition among low-income group of this country and such kind of biohazards not only disrupt their nutritional value of food fishes, it also disrupts the ecological balance. The present study, is aimed to investigate Blood performance and biochemical studies in the Liver, Kidney and Gill tissue of *Heteropneustes fossilis* (Bloch) *(e.g. H. fossilis)* exposed a potential pesticide Pyrethroid. Different hematological parameters were determined by using blood samples from control and treated groups and analyzed. For enzymatic study tissues like Live, Kidney and Gills obtained from both control and treated groups were analyzed by using enzyme kits. Student's t test is use to compare the statistical data. Using MS excel all data are interpreted in bar diagram. Altered blood cell count, increased value of aberrant cell count, increased amount of micronuclei formation was found due to the effect of Pyrethroid. Significant increase in ACP and Glutathione-S-Transferase (GST) activity was recorded in the result. In the recovery period the GST values were recorded almost normal like the control group. The hazardous effect of pyrethroid was seen in both cellular level and enzymatic level in this study and found that it can disturbed normal blood physiology as well as behavioural and enzymatic stability.

Keywords: Micronuclei, Haematotoxicity, Acid phosphatase, Glutathione-S-transferase, *H. fossilis.*

INTRODUCTION

Fishes are considered as a specific indicator of water pollution (Plessl *et al.,* 2017). From polluted water and food their body accumulated chemicals by the process of biomagnifications (Daoud *et al.,* 2022). Due to biomagnification the toxicity travels to the body of human consumers (Al-Ghasis and Ali 1999; Ali and Sreekrishanan 1999). Different hematological, biochemical and antioxidant enzyme measurement in organism give information about the hazardous effects of organisms exposed to a certain amount of chemicals. Such analysis provides early signals of toxicological effects (Livingstone, 1995). Different industries like pharmaceutical, textile chemical, plastic and paper mills etc. produces and release different types of chemicals, for example-heavy metals, insectisides, plasticizers etc. and such chemicals can create hormonal and organ dysfunction (Rehma *et al.,* 2021; Vieira *et al.,* 2021).

Chrysanthemum cinerariaefolium and *Chrysanthemum cineum* are plants from where a natural compound be extracted called as pyrethrum. The synthetic form of pyrethrin is called as pyrethroid. Pyrethroids are highly used insecticides now-a -days. Pyrethroids are highly toxic and less degradable to environment. The manufacturing companied formulated pyrethroids with other compounds like piperonyl sulfoxide, piperonyl butoxide for making insecticides. This formulation makes the compound more toxic (Thatheyus *et al.,* 2013)

Haematological and haemato-biochemical study is one of a very effective way to diagnose fish health condition. This study included parameters like RBC, WBC, haemoglobin, and haemocrait (Esmaeili, 2021). This kind of study can provide us information about any kind of health abnormalities due to hazardous effect of contaminated water.

A micronucleus test is a test used to find out in potential genotoxic compounds in food and habitat of an organism. The presence of micronuclei in cell provides the information about the organism's exposure to certain chemicals that cause spindle formation and

that leads to the formation of micronuclei. Micronuclei are clumps of cytoplasmic chromatin that are formed when chromosomes are fragmented or fail to be incorporated into the cell nuclei during anaphase of cell division. The assay is the recognized as one of the most successful reliable assays for genotoxic materials (Esmaeili, 2021; Ahmed *et al.,* 2014).

Acid Phosphatase (ACP) is an enzyme produced by lysosome. When it rises in the body, it indicates cell damage (Novikoff, 1961). Glutathione-S-transferase (GST) is an enzyme produce by cell and its main function is detoxification of toxic metabolites and environmental contaminants (Egaas *et al.,* 1995). GST take part in detoxification of reactive oxygen species during oxidative stress (Rudneva *et al.,* 2010).

It is reported that contaminated water by industries or by anthropogenic effect alters the histology and physiology of fishes (Fanta *et al.,* 2003; Pathan *et al.,* 2021). Enzymes with antioxidant activity of fishes play a deciding role in balancing cellular feedback mechanism and therefore this study have been very eye-catching for eco-toxicologist since oxidative damage was reported as a mechanism of toxicity in aquatic animals live with environmental contaminants which are released in the water bodies by different industries (Satos *et al.,* 2004).

The present study aims to evaluate the Blood performance, Micronuclei study and potential action of ACP and GST indifferent tissues of *H. fossilis* like liver, gill and kidney after treated with Pyrethroids.

MATERIALS AND METHODS

Collection of fish. Air breathing Cat fish *Heteropneustes fossilis* (Bloch) was selected for the study and the fish were collected (40-60 g weight and 12-25 cm in length) with the help of local fisherman from a pure fresh water body named Charon Beel, (Kalita *et al.,* 2006) of Morigaon district, Assam.

Chemicals. All the chemicals are purchased from HiMedia Lab. Pvt. Ltd and supplied by Northeast chemicals (Panbazar) Guwahati, Assam, India.

LC 50 study. Fishes were acclimated in aquaria for one week prior to the experiment and then released them into five aquaria having 10 individuals of each with different concentration (6.25, 12.5, 25, 50, 100%) of pyrethroid treated water for determination of LC_{50} (Ipsen and Feigl 1970). The lethal concentration was determined from the study in a concentration of 6.25mg/l (Kaur and Dua, 2015). The behaviour alteration of the treated fishes was also determined during this study.

Haematological studies. Using the standard method of Jain (1986) the blood parameters are determined. Different parameters like RBC count, WBC count, Haematocrit volume and haemoglobin estimation was determined in this present study. The blood collection was done by using heart puncture method and the blood were then transferred into a tube pre coated with anticoagulant. For RBC and WBC count haemocytometer was used and for detection of haematocrit level centrifugation technique for 5 mints at 9000 rpm was done using microcentrifuge machine. For haemoglobin estimation Sahli's haemoglobinometer is used (Jain, 1986).

For micronuclei study blood smear was prepared in an microscopic slide. Then methanol was added to fix the smear for 10 mints. After drying the smear was stained using 6% Giemsa stain. Using a binocular microscope, 100x lance (Labomed), about 1000 erythrocytes were examined to detect the presence of micronucleated (MNC) and aberrant cells (AC) for both treated and control group. The RBCs having nuclear and cytoplasmic abnormalities are marked as AC (Nwani *et al.,* 2010). Notched, lobed, deformed and elongated nuclear structure was considered as nuclear abnormalities and for cytoplasmic abnormalities vacuolated cytoplasm and swelled cells were considered.

Enzyme study

Determination of acid phosphatase activity

ACP activity was determined by using the modified method of (Gomori, 1941). To perform ACP activity, five aquaria for rearing *H. fossilis* were used. The first group was allowed to expose to the normal water as control and other three groups were exposed to sublethal concentration (50-90%) of pyrethroid treated water for 30, 60 and 90 days. The fishes were sacrificed for liver and gills for each period of study.

Determination of glutathione -stransferase activity:

To perform the experiments on GST activity 30 fishes were accustomed for 15 days. 10 accustomed fishes were kept in normal water and grouped as control and 20 fishes were exposed to sub lethal concentration of pyrethroid treated water (50-90%) and grouped as treated group. To estimate GST level 10 individual from each control and treated groups taken and tissues (Liver, gill and kidney) were removed. At the same time the remaining 10 fishes from treated group were kept in normal water to study the recovery for 15 days. After recovery period over the fishes were sacrificed and GST levels were again studied by removing tissues (liver, gill and kidney) form the recovery group of fishes.

RESULTS AND DISCUSSION

From result of LC50 study it was documented that 12.5mg/l pyrethroid showing 50 % mortality (Table 1) after 96 h of exposure. The behavioral alteration on pyrethroid exposed fish groups is presented in Table 2. Fishes showed vigilance and ceased swimming, hyper excitation and erratic movement when the concentration of pyrethroids increased. The amount of mucous secretion is also increased on increasing amounts of pyrethroids concentration. In the haematological study, treated group showed notable alteration in different parameters after 96 h of exposure. The Hb count, RBC count and Hematocrit volume showed significant declining in compare to the control grops. 7.02 Hb level were found at 96 h of exposure.

Table 1: Determination of LC 50 value.

Fish	Control	6.25mg/l	12.5mg/l	25mg/l	50mg/l	100mg/l
H. fossilis	No Mortality (NM)	NM	LC_{50}	60% mortality	75 % mortality	100% mortality

Table 2: Impact pyrethroids on behaviour of fish *H. fossilis* after exposed for 96 h.

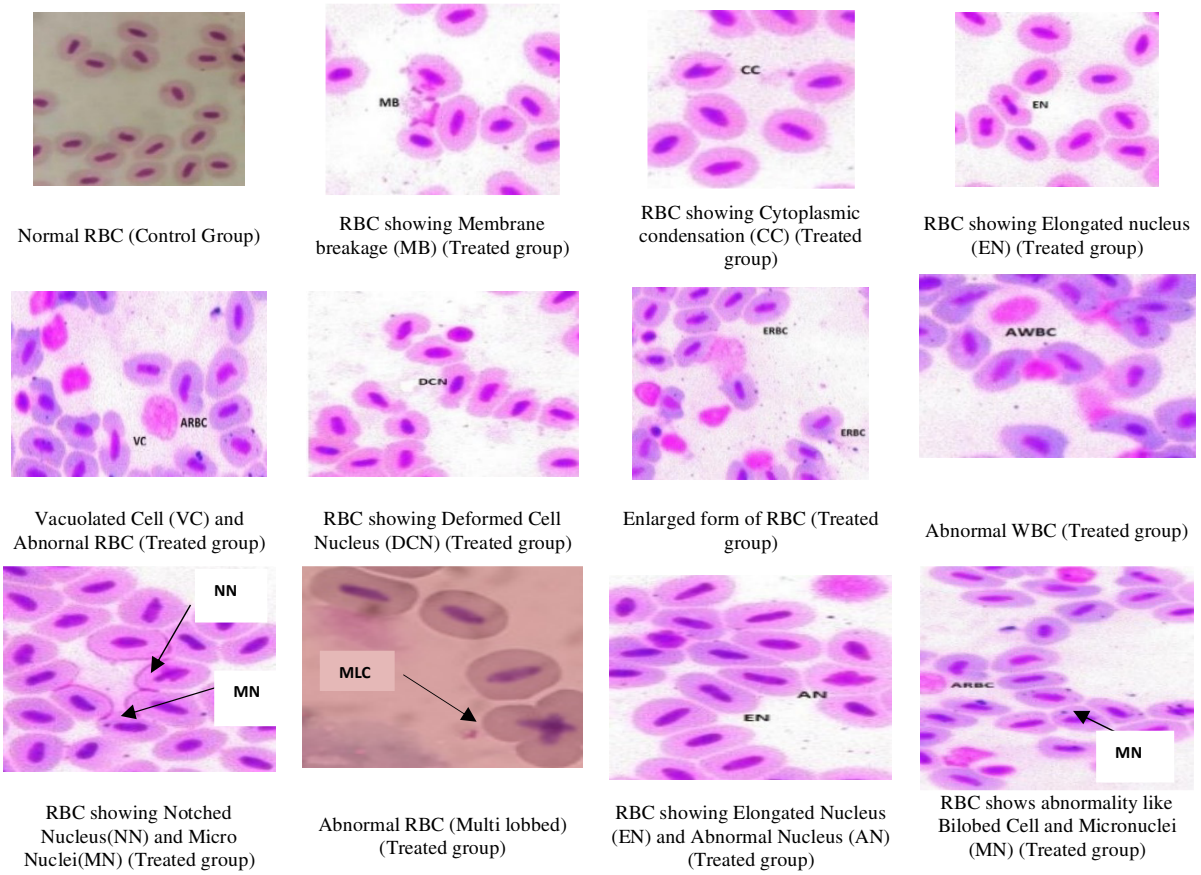
Parameters	Control	6.25mg/l	7.25mg/l	8.25mg/l	10.25mg/l	12.25mg/l
Vigilance	_	_	+	+	++	+
Ceased swimming	_	_	+	++	+++	_
Hyperactivness	+	+	_	_	_	+++
Loss of balance	_	_	_	+	+	++
Rate of swimming	+	+	++	+	+	+
Rate of opercular activity	+	+	++	++	+++	+++
Pigmentation	_	_	_	_	+	++
Mucous secretion	_	_	_	+	++	+++

(-) None, (+) Mild, (++) Moderate, (+++) Strong

From control group the value is decreases 19.40%. RBC and hematocrit values were decreases as well. For RBC the value is decreases from 2.49 in control to 1.96 after exposure. Hematocrit level declines from 42.15% to 27.19 %. The WBC count was increased in treated groups and at 96 h the highest amount of WBC count was recorded *i.e,* 10.92. The WBC count showed 1.22-fold hike compared to control group. The count of different WBC molecules like neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil were done separately and showed significant increase (Table 3).

The morphology of Micronucleated (MNC) and Aberrant cell (AC) is given in Fig. 1. The study showed the significant increase in MNC and AC after treatment with Pyrethroids as compared to the control groups. In the control group, MNC and AC frequency was very less whereas in the 96h treated group significant dose dependent increase was observed Fig. 2.

After exposure to 3.81 mg/l pyrethroids the number of micronucleated cell increases from 0.12 ± 0.043 to 0.81 ± 0.01, and the percentage of aberrant cells increased from 7.53 ± 0.64 to 43.89 ± 1.24 (Mean ± S.E.). Thus, in the result significant effect was shown with the rise in the concentration and time of exposure ($P < 0.05$).



Normal RBC (Control Group)

RBC showing Membrane breakage (MB) (Treated group)

RBC showing Cytoplasmic condensation (CC) (Treated group)

RBC showing Elongated nucleus (EN) (Treated group)

Vacuolated Cell (VC) and Abnormal RBC (Treated group)

RBC showing Deformed Cell Nucleus (DCN) (Treated group)

Enlarged form of RBC (Treated group)

Abnormal WBC (Treated group)

RBC showing Notched Nucleus(NN) and Micro Nuclei(MN) (Treated group)

Abnormal RBC (Multi lobbed) (Treated group)

RBC showing Elongated Nucleus (EN) and Abnormal Nucleus (AN) (Treated group)

RBC shows abnormality like Bilobed Cell and Micronuclei (MN) (Treated group)

Fig. 1. Showing different cytoplasmic and nuclear abnormalities in blood cell of *H. fossilis* after exposure of pyrethroid.

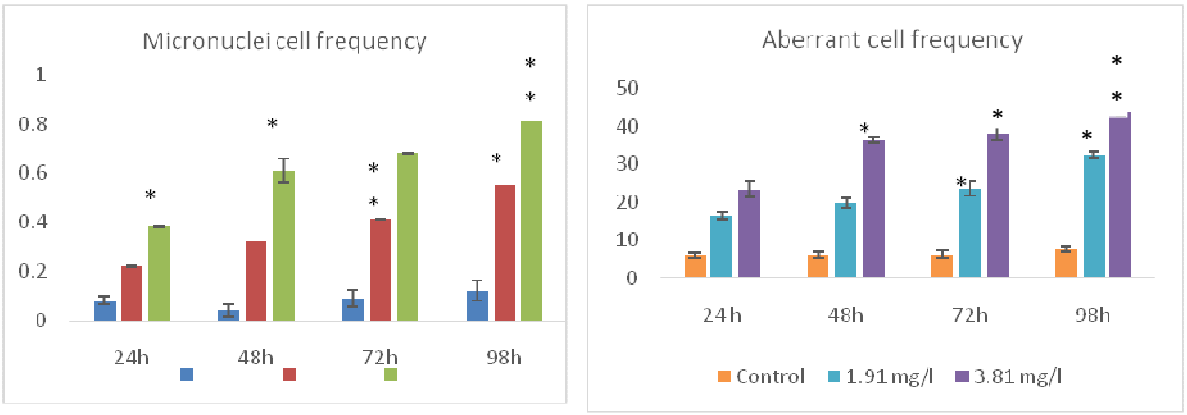


Fig. 2. Micronuclei cell frequency and aberrant cell frequency in pyrethroid treated groups.

Table 3: Blood parameters of (96 h treated group comparing with control group).

Parameters	Control	96 h treated
Hb content	8.71±0.94	7.02±3.24*
RBC count	2.49±0.76	1.97±2.62*
WBC count	9.7±0.23	10.92±1.91
Neutrophils	35.00±0.44	36.00±2.56*
Lymphocyte	58.00±0.89	59.00±3.11*
Monocyte	2.00±0.21	3.00±0.001
Eosinophils	3.00±0.11	2.00±0.002
Basophils	2.00±0.09	2.00±0.09
Haemocrait	42.15%	27.19%

(MEAN±SEM,* P<0.005)

The morphology of blood cells of control as well as exposed group is shown in Fig. 3. The morphological abnormalities od RBCs such as irregular shaped, cytoplasmic bleb, notched, elongated cells, and fused cells were seen in the treated groups.

In the acid phosphatase activity study liver showed higher ACP activities than gills with increasing period of exposure. The increased ACP activity may be due to enhanced enzyme turn over under pyrethroid's stress. The changes altered due to toxicity of pyrethroids in *H. fossilis* and resulted in elevation of ACP activity in different tissues (liver and gills). Significant increase in ACP activity after 90 days of exposure at 22.5 ppm concentration was higher than 30 days exposure (Table 4).

Table 4: Acid Phosphatase Activity (ACPA) in pyrethroid exposed fish.

Sample	Normal/Control	30 days exposed fish	60 days exposed fish	90 days exposed fish
Liver-a	ACPA $^{0-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$	ACPA $^{3-4}$
Liver-b	ACPA $^{0-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$	ACPA $^{3-4}$
Liver-c	ACPA $^{0-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$	ACPA $^{3-4}$
Gill-d	ACPA $^{0-1}$	ACPA $^{1-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$
Gill-e	ACPA $^{0-1}$	ACPA $^{1-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$
Gill-f	ACPA $^{0-1}$	ACPA $^{1-1}$	ACPA $^{1-2}$	ACPA $^{2-3}$

ACPA 0-1: No activity, ACPA 1-2: Very low activity, ACPA 2-3 Moderate Activity, ACPA 3-4: Higher activity.

In the result of GST value in exposed period increased percentage of GST was recorded *i.e,* 1.1% in liver, 1.26% in gills and 1.26% in kidneys (Fig. 3 A, B, C, D). In normal liver the GST value was recorded between 48.29 to 48.61 IU/mg with an average value of 48.40± 0.07 IU/mg. But after 15 days post treatment it was recorded between 50.05 to 50.57 IU/mg with an average of 50.290.02 IU/mg. Post treatment, the gills showed value between 15.89 to 16.55 IU/mg with an average value of 16.23±0.13 IU/mg and in kidneys it was 31.91 to 33.67 IU/mg with an average value of 33.20±0.12 IU/mg.

In the recovery period after 15 days post recovery the 48.31 to 48.98 IU/mg with mean value 48.88± 0.033 IU/mg. In gills the values were between 15.67 to 15.91 IU/mg with an average value of 15.80±0.04 IU/mg. and for kidneys the average value was 31.96±0.18 IU/mg and ranged between 31.64 to 32.09 IU/mg. When comparing the data of normal and recovery period, it showed similarities.

Pyrethroids are chemicals which can affect DNA integrity and it may analyses by micronuclei assay. The percentage of micronucleated cells and percentage of aberrant cells are the parameters which are analyzed to detect DNA damage. After every exposed period the level of micronucleated cell frequency and aberrant cell frequency was noticed (P<0.01) in the study. In highest concentration and highest duration (96h) maximum micronucleated frequency *i.e,*0.81±0.001 and aberrant cell frequency *i.e,*43.89±1.24 was noticed. Using Yadav and Trivedi (2009) method, this study was conducted. In their work duration dependent increase in micronucleus frequency in fish exposed to heavy metals was detected. In their result Haemato-cellular structure

like lobed nuclei, blebbed nuclei, notched nuclei, micronuclei were found in *Orechromis niloticus* when exposed to heavy metal (Matsumoto *et al.,* 2006). Formation of nuclear abnormalities like lobed nucleus, nuclear buds, elongated nucleus in fish blood cells if exposed to certain chemicals were may be due to the hostile effects by hazardous chemical causing pollution. All these changes obstruct the chromosomal attachment due to gene amplification and leads to the emergence of notched, budded, binucleated, and other distorted nuclei (Bolognesi *et al.,* 2006; Ergene *et al.,* 2017).

Liver is the prime center for removing xenobiotic and biocides in organisms (Croom, 2012). The metabolic pathways in liver of fish are affected by various pollutants due to the alteration of cellular enzymatic activities. The increased and decreased activities of ACP indicate disturbance in the structure and integrity of cell organelles like endoplasmic reticulum and membrane transport system (Renuka, 2019).

Acid phosphatase are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign material, hence it has a role in certain detoxification function. Increase in Acid phosphatase enzyme activity might be due to increase in protease activity which cause damage to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytoplasm (Bujjamma and Padmavathi 2008). Aquatic organism like fishes has developed some antioxidant enzyme in their body to get rid of toxic compounds accumulated in their body and GST is one of a such enzymes. GST considered as phase II biotransformation of xenobiotic and carcinogens (Roy, 2002; Voso *et al.,* 2008). In the study, the decreasing amount of GST in the exposed group after recovery period could be a part of this xenobiotic metabolism process.

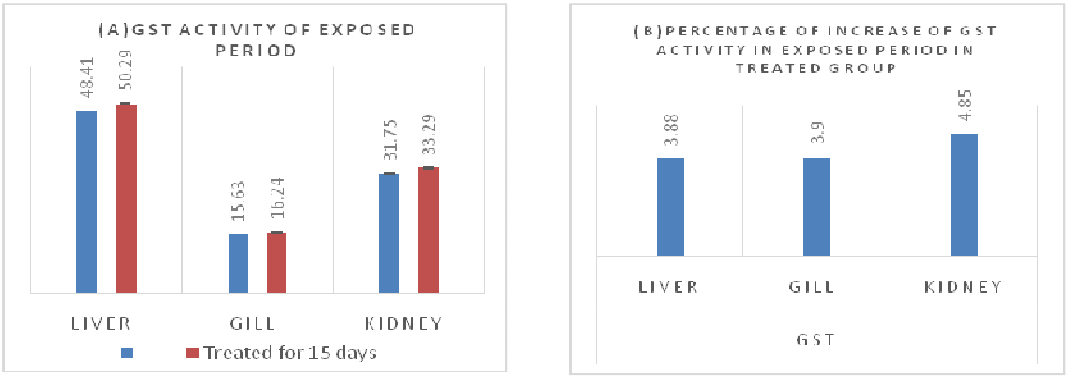


Fig. 3. Figure showing GST activity and percentage of increase in exposed period (A, B).

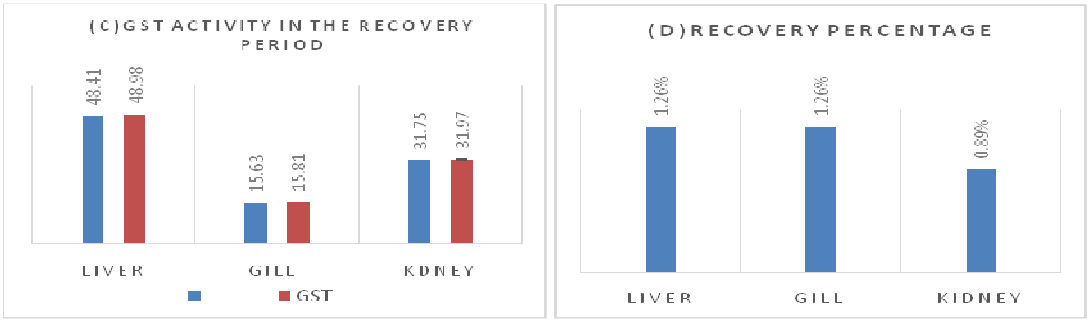


Fig. 3. Figure showing GST activity and recovery percentage in the recovery period (C, D).

CONCLUSIONS

Water pollution due to the use of agricultural and industrial insecticides are very common now a days. These uses benefitted the farmer for only a particular period of time. But the hazards produced by this chemical remain in the environment for decades and harming the ecosystem and showing health hazards to the aquatic organism. Due to biomagnification this hazard transferred to the human bodies too. Therefore, it is the high time to opt for new biodegradable, high effective and low hazardous component for our agricultural as well as industrial yield.

FUTURE SCOPE

This study is just a preliminary evaluation of the hazardous effect of pyrethroids. Genetic level study my give us more information about its genotoxicity. In future this kind of study can be an eye opener for the world to lower the use of such chemicals and make environment friendly materials as an option.

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Conflict of Interest. None.

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