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# Biochemical and Histological Effects of Glyphosate on the Liver of *Cyprinus carpio* (Linn.)

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**Abstract:** Glyphosate is an active ingredient of Roundup®, a herbicide which is extensively used for agricultural applications and control of vegetation in non-crop areas often leading to its runoff from sites of application into water bodies posing serious threat to non-target species including fish. The present investigation was undertaken to evaluate the biochemical and histopathological effects of glyphosate on the liver of freshwater fish, *Cyprinus carpio* (Linn.) after calculating the 96 h $LC_{50}$ of glyphosate (Roundup®41% SL) which was 3.260 ppm. The fish fingerlings having mean wt. 3g ±0.5 and mean length 5.5cm ±0.35 were exposed to two sublethal concentrations of glyphosate *i.e.* 25% of $LC_{50}$ ($T_1$) and 50% of $LC_{50}$ ($T_2$) for a period of 28 days. Total soluble proteins, lipids and enzymatic activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were recorded at weekly intervals and significant ($p>0.05$) decrease in protein and lipid content of the liver was continually observed till the termination of the experiment. However, the enzymatic activities of AST and ALT in liver showed a significant ($p<0.05$) increase with increasing concentrations of glyphosate and duration of exposure. The histo-morphology of liver in fish exposed to glyphosate exhibited vacuolation of hepatocytes, pyknotic nuclei, degeneration of cytoplasm, and infiltration of leukocytes, necrosis and severe vasodilation in the treatments. The severity of biochemical and histological alterations was more pronounced in $T_2$ after 28 days of exposure. The increase in activities of AST and ALT and the decrease in protein and lipid content of the liver following exposure of fish to the herbicide suggest enhanced protein catabolism, hepatocellular damage and increased utilization of energy stores to compensate for higher energy demands during stress. This indicates that the above said herbicide causes potential harm to the aquatic life.

**Keywords:** *Cyprinus carpio* L., Glyphosate, Histology, Lipids, Liver, Proteins, Transaminases

## 1. Introduction

Water pollution is a major problem of this century owing to the addition of various pollutants through many ways that change the natural qualities of water (Voltz *et al* 2005) which adversely affect the non-target organisms and lead to their mortality upon acute and chronic exposure (John 2007, Sarwar *et al* 2007, Velcheva *et al* 2012, Sabae et *al* 2014). In case of inland water contamination, pesticides are known to contaminate areas closer to their application which affect growth, reproduction and nutritional value of fish, when their concentration in water exceeds the critical maximum limit (Moore and Waring 2001). The use of agrochemicals in Indian agriculture is growing due to intensive agricultural inputs, which coupled with careless handling, accidental spillage, surface runoff from sites of application into natural water-ways may contribute long term effects in the environment (Cavas and Konen 2007).

Glyphosate, marketed by the trade name Roundup®, is a broad-spectrum, non-selective herbicide used for inhibition of unwanted weeds and grasses in agricultural, industrial, urban, forestry and aquatic landscapes (Cavas and Konen 2007). It is a highly water-soluble substance (10500 mg/l) with a half-life of about 3.5 to 90 days and in addition contains, a cationic surfactant denominated polyoxyethylamine (POEA) that confers toxicological properties different from those of GP (Folmar *et al* 1979).

Glyphosate-based herbicides are known to be hazardous to the aquatic environment and are toxic to aquatic life with long lasting effects (Sihtmae *et al* 2013, Tomlin 2006).

Fish species have been reported to be most sensitive to aquatic pollutants during their early life stages (Jiraungkoorskul *et al* 2003) and the biochemical parameters in fish liver are considered sensitive for detecting potential adverse effects of pollutant damage. Liver micromorphometry has been found to be a reliable biomarker of toxic damage because histological and ultrastructural changes in the cells can be used to predict pollutant stress in acute and chronic concentrations changes in the tissue of individual organisms (Stentiford *et al* 2003). The liver is generally regarded as central organ of xenobiotic metabolism in fish and is a target organ affected by toxicant exposure (Mohamed 2009).

These criteria were the reason to conduct the present study on biochemical and histopathological effects on common carp (*Cyprinus carpio* L.) to determine possible adverse effects of aquatic herbicides.

# 2. Material and Methods

### *2.1. Procurement and Maintenance of Fingerlings of Common Carp*

Fingerlings of common carp (*C. carpio* L.) having mean wt. 3g ±0.5 and mean length 5.5 cm ±0.35 were procured from College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and acclimatized to the laboratory conditions in large plastic tubs of 100 litres capacity for about a fortnight prior to the initiation of the experiment. The fishes were fed with pelleted feed twice a day @ 1.5% of biomass twice a day; the tubs were continuously aerated with electrically operated aerators (2 aerators/ tub) and water was filtered (2 filters/ tub) to clear the faecal matter from the water. The experimental protocol met the OECD guidelines for testing of chemicals.

### *2.2. Biochemical Studies*

Fingerlings of common carp of approximately same size were exposed to two sublethal concentrations of glyphosate (Roundup®) *i.e.* 25% of $LC_{50}(T_1)$ and 50% of $LC_{50}$ ($T_2$) for 28 days. Five fish specimens were collected at 7days interval from each of the experimental and control groups. The fish were anaesthetized with MS 222 (tricaine methane sulphonate), and sacrificed by decapitation and liver was collected for biochemical analysis of total proteins, lipids and amino transferases (AST and ALT).

### *2.3. Estimation of Total Proteins*

Biochemical parameters were studied in liver of fingerlings of common carp. 0.5 g of livertissue were homogenized in 2 ml of phosphate buffer saline (PBS: 0.1M, pH 7.4) and centrifuged at 3000 rpm for 10 min. The supernatant was used for the estimation of total proteins by method of Lowry *et al* (1951). The absorbance was read at 520 nm in a spectrophotometer against reagent blank. A standard curve was prepared by taking Bovine serum albumin (BSA) solution in range of 20-200 µg / ml.

### *2.4. Estimation of Aminotransferases*

Aspartate transferases (AST) and Alanine transaminase (ALT) activities were assayed in liver by colorimetric method of Reitman and Frankel (1957) as described by Bergmeyer (1974). Standard curve was prepared with working pyruvate standard solution (iii) and the absorbance was determined at 510 nm.

### *2.5. Estimation of Total Lipids*

Total lipids were extracted from the liver by the method of Folch *et al* (1957). The dissolved total lipids so obtained were evaporated to dryness in a pre-weighed crucible. The initial and final weights of crucible were noted. The difference in weights gave the total lipid content.

### *2.6. Histopathological Studies*

Liver of fishes were cleared of the adhering tissues and delete 'representative' samples were fixed in aqueous Bouin's delete 'fixative' for 24 hour.

After complete fixation, the tissue was processed, sectioned and stained by following standard histological techniques of dehydration, clearing and embedding in paraffin wax (melting point between 58-60°C) (Humason 1967). Serial sections of5-7 µm thick were cut with the help of microtome and stained in haematoxylin-eosin protocol and mounted in DPX. The slides were observed under microscope and compared with the slides from control group for the determination of histological alterations.

### *2.7. Statistical Analysis*

Probit analysis (Finney, 1971) was used to calculate the median lethal concentration and time with their upper and lower confident limits.

All statistical comparisons for biochemical analysis of proteins, lipids and aminotransferases (AST and ALT) were presented as mean ± standard error of mean (S.E.M). Comparisons were made between control and concentrations *i.e.* 25% of $LC_{50}$ and 50% of $LC_{50}$ and also between no. of days of exposure using Bivariate ANOVA (Factorial CRD) in CPCS 1. A "p" value of 0.05 was selected as a criterion for statistically significant differences.

# 3. Results and Discussion

The 96 hour $LC_{50}$ and $EC_{50}$ values of glyphosate (Roundup®) were 3.260 ppm and 0.509 ppm, respectively. Normal behavior was observed in control, whereas, restlessness, lack of balance and coordination, gulping of air, frequent surface to bottom movement and erratic swimming were recorded in different concentrations of the herbicide (Plate I, Figure 5a & 5b). Similar observations were recorded by earlier Aguigwo (2002) and Omoniyi *et al*

(2002). In addition, Rahman *et al* (2002) also observed rolling movement, sudden fast movements, swimming on back and resting at the bottom when fish was exposed to herbicide contamination. This indicates respiratory impairment, probably due to the presence of toxicant glyphosate in water which affects the first point of contact, the gills.

### *3.1. Biochemical Studies*

#### *3.1.1. Proteins*

The results revealed a significant decrease ($p>0.05$) in the protein content of liver with an increase in concentration of glyphosate (Roundup®). At 7 days of exposure, the protein content of liver in treated group $T_1$ (3.832±0.15 mg/g) and $T_2$ (2.545±0.13 mg/g) decreased significantly ($p>0.05$) as compared to control (8.704±0.44 mg/g). Similarly, at 14 days of exposure, the level of protein content in liver of treated group $T_1$ (2.076±0.20 mg/g) and $T_2$ (1.419±0.12 mg/g) was significantly ($p>0.05$) lower than the control group (9.782±0.16 mg/g). This trend in the decrease of liver protein content continued till the termination of the experiment. Further, the decrease in protein content recorded in treated groups $T_1$ and $T_2$was in direct proportion to the increase in period of exposure as it increased with exposure time (Figure 1).

In the present study the decline in protein content of liver may be attributed to decrease in protein synthesis following exposure to the herbicide and/or due to increased protein catabolism. The results are in accordance with the findings of Singh and Bhati (1994) who too, reported a decrease in protein content in the liver of *C. punctatus* with increase in period of exposure of fish to 2,4-D (herbicide).

Similarly, Singh *et al* (2015) observed a decrease at 24 and 36 days of exposure in level of total protein in serum of *C. carpio*when exposed to dimethoate at 0.96mg/l. Ganeshwade *et al* (2012) too, recorded a decreased protein content in testis, ovary, brain, liver, gills and muscles of *Puntius tictow*hen exposed to dimethoate. The decrease in protein content may be increase in rate of degradation of proteins to amino acids which are probably sent to TCA cycle through aminotransferases so as to meet the high energy demands during metabolism in the stress conditions. According to Shinde *et al* (2002) the decrease in the protein content of liver has been hypothesized to its utilization for metabolism due to increase in proteolytic activity the decrease observed in protein content is definitely attributed due to stress conditions that cause damage to the hepatic tissue and enhance proteolysis leading to depletion in protein contents affecting the nutritive value of fish (Hilmy *et al* 1985); noted a decline in protein content of liver, brain and muscle tissues of *C. carpio* following exposure to 10 µg/lt. of fenvalerate (Reddy *et al* 1991). Similarly, researchers have confirmed a decline in protein level of liver, muscles, gills and brain of *Labeo rohita* following exposure to carbamide Rajyashree (1996), and the changes in protein content of liver have been correlated with alterations in structure of liver and hepatic cords leading to changes in metabolism of liver. However, Begum (2004) and Crestani *et al* (2006) reported an increase in liver protein content of *Clarias batrachus* after exposure to carbamate, and also to a herbicide, clomazone in silver catfish, *Rhamdia quelen*, respectively. The response was, therefore, species specific with catfishes responding differently.

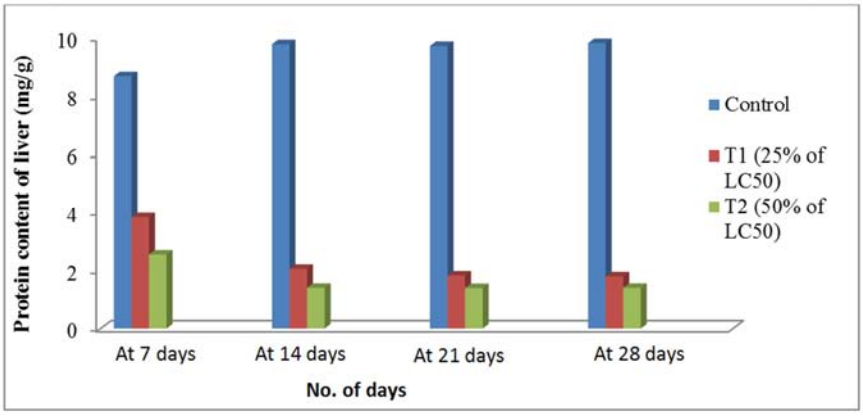


***Figure 1.*** *Effect of sublethal concentrations of glyphosate on the protein content of liver (mg/g) in Cyprinus carpio (Linn.) fingerlings.*

#### *3.1.2. Lipids*

The results revealed a significant ($p>0.05$) decrease in the total lipid content of liver with the increase in concentration of glyphosate (Roundup®). At 7 days of exposure the total lipid content in the treated groups $T_1$ (0.645±0.05 mg/g) and in $T_2$ (0.55±0.03 mg/g) declined significantly ($p>0.05$) as compared to the control (0.677±0.09 mg/g). Similarly, at 14 days of exposure, the level of lipid content in liver of treated groups $T_1$ (0.322±0.08mg/g) and $T_2$ (0.236±0.13mg/g) was significantly ($p>0.05$) lower than the control group (0.629±0.07mg/g). This decrease of lipid content continued till the termination of the experiment (Figure 2). Further, as in case of proteins the decrease in lipid content of liver in treated groups $T_1$ and $T_2$ increased with increase in period of exposure. Our results are in accordance with the observations of a decline in lipid content of liver in *Tilapia mossambica* when subjected to atrazine and to Monocrotophos

respectively as recorded by Sreenivasa (2002) and Remia *et al* (2008) respectively. Recently, Pechiammal and Kiruthika (2016) also recorded a decrease in lipid content of liver in freshwater fish, *Cirrhinus mrigala* following exposure to an insecticide, Rogor. These authors attributed the decrease to the utilization of energy store to meet more energy demands for detoxification process and also to balance the hindrance of normal metabolism.

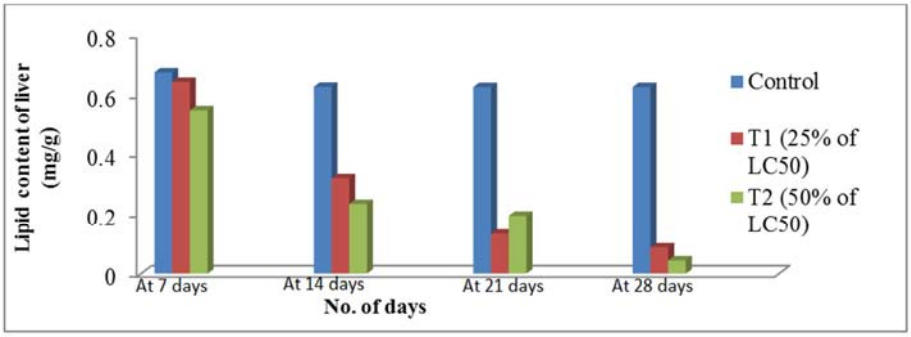


***Figure 2.*** *Effect of sublethal concentrations of glyphosate (Roundup®) on lipid content of liver (mg/g) in Cyprinuscarpio (Linn.) fingerlings.*

### *3.1.3. Aminotransferases*

*Aspartate amino transferase (AST)*

The results have revealed that enzymatic activity of aspartate amino transferase (AST) in liver showed a significant ($p<0.05$) increase with increasing concentrations of glyphosate (Roundup®). The enzymatic activity of AST at 7 days of exposure in treated groups $T_1$ (40.714±0.41 IU/lt.) and $T_2$ (76.552±0.40 IU/lt.) was noted to be significantly ($p>0.05$) higher than that recorded in the control (0.101±0 IU/lt.). Similarly, at 14 days of exposure, the enzymatic activity of AST in $T_1$ (48.378±0.28 IU/lt.) and $T_2$ (94.546±0.39 IU/lt.) was significantly ($p>0.05$) higher when compared with the control group (0.103±0 IU/lt.). This trend in the increase of enzymatic activity of AST in the treated groups was observed during the entire duration of the experiment (Figure 3).

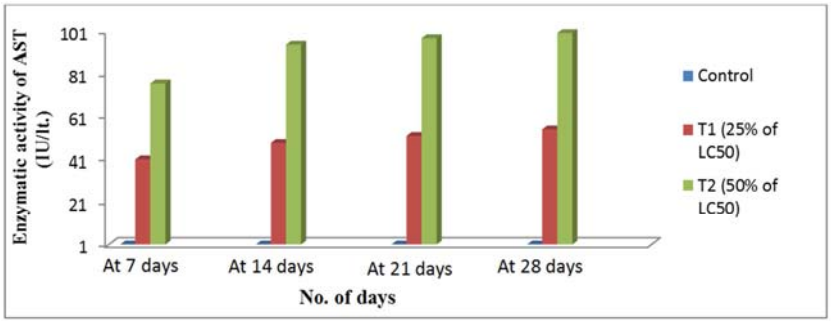


***Figure 3.*** *Effect of sublethal concentrations of glyphosate (Roundup®) on the enzymatic activity of AST (IU/lt.) in liver of Cyprinuscarpio (Linn.) fingerlings.*

The observations are supported by the increased activities of ALT, AST and Alkaline phosphatase following exposure of *O. niloticus* to glyphosate at 5 ppm and 15 ppm concentrations of Roundup (Jiraungkoorskul *et al* 2003). Similar observations were recorded by Neelima *et al* (2013) who recorded an increase in the activity of AST in gills, muscle and liver of *C. carpio* exposed to cypermethrin.

In the present study, the increase in enzyme activity (AST) may be attributed to enhanced transamination for transfer of more amino acids into Tricarboxylic Acid (TCA) cycle. Since, the transaminases are located in mitochondria and are useful biomarkers of liver injury, the alteration in transaminases activity can also be associated with mitochondrial disruption and tissue damage as a consequence of glyphosate exposure.

According to Webb (2001) animals reduce feeding under stress conditions, so additional energy is required to overcome stress conditions which is made available by the animal from the of transaminases increase with proteolytic activities (Ganesh *et al* 2006). The enhanced activity of transaminases may signify that fishes can use free amino acids from amino acid pool for generation of energy, so during dysfunctioning the transaminases helps the cells to switch to gluconeogenesis (Naveed *et al* 2004; Naveed *et al* 2010).

In contrast, Begum (2004) reported a decline in the levels of AST in the liver of fish *Clarias batrachus* (Linn.) under the influence of Carbofuran. This again reflects the different response to toxic stress by carps and catfishes.

*Alanine amino transferase (ALT)*

The results revealed that the enzymatic activity of alanine aminotransferase (ALT) in liver showed a significant increase ($p<0.05$) with increasing concentration of glyphosate (Roundup®). At 7 days of exposure the enzymatic activity of ALT in $T_1$ (41.838±0.12 IU/lt.) and $T_2$ (104.45±0.33 IU/lt.) was noted to be significantly ($p<0.05$) higher than that

recorded in the control (0.28±0 IU/lt.).

Similarly, at 14 days of exposure, the enzymatic activity ALT in $T_1$ (49.28±0.36 IU/lt.) and $T_2$ (105.574±0.10 IU/lt.) were significantly ($p>0.05$) higher than the enzymatic activity of ALT in the control group (0.267±0 IU/lt.). This trend in the increase of enzymatic activity of ALT continued till the termination of the experiment. However, in the control the enzymatic activity was observed to initially decreased at 14 days of exposure and increased subsequently. Further, the enhanced activity of ALT was noticed in both $T_1$ and $T_2$ with increase in the period of exposure (Figure 4).

In the present investigation, the rise in enzymatic activity of alanine amino transferases (ALT) with increase in concentration and exposure period of herbicide may be assigned to more active transamination and more synthesis of the enzyme under stressful conditions of the animal which supported gluconeogenesis for more energy production, the enhanced enzymatic activity is also supported by the breakdown of hepatocytes following exposure to glyphosate (Roundup®) as observed in the present study.

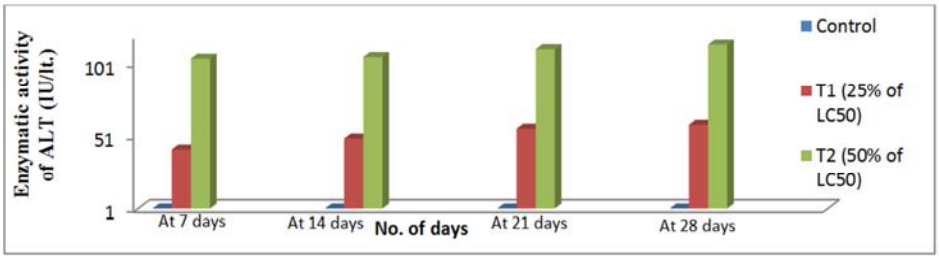


***Figure 4.*** *Effect of sublethal concentrations of glyphosate (Roundup®) on the enzymatic activity of ALT (IU/lt.) in liver of Cyprinus carpio (Linn.) fingerlings.*

Our observations are in agreement with the results of Yousafzai and Shakoori (2011) who studied that in fish liver the enzymatic activities were enhanced due to increased synthesis of enzymes to overcome the stress caused by toxicants. On the contrary decline in the activities of enzymes may be related to decrease in synthesis of enzymes and it can also be attributed to alterations in permeability of liver cells. This can be as a result of induction or inhibition of enzymes during stress.

Similarly, Neskovic *et al* (1996) reported an increase in enzymatic activities of ALT and AST in common carp following exposure to glyphosate. The increase in the transaminases signifies the use of amino acids for gluconeogenesis and is indicator of liver damage (Philip *et al* 1995). Elevated activities of AST and ALT were also studied in *Channa punctatus* after exposure to monocrotophos (Agrahari*et al* 2007). Banae e *et al* (2012) too, stated that during stress the enhanced AST and ALT enzymatic activities arouse the gluconeogenesis process because these enzymes play an important role in the L-amino acids mobilization.

In contrast, as in earlier responses, Begum (2004) reported a decline in the level of ALT in the liver of fish *Clarias batrachus* (Linn.) under the influence of carbofuran, and interestingly Li *et al* (2004) recorded a decrease in the activities of AST and ALT in liver of the fish rainbow trout after a chronic exposure to carbamazopine.

### *3.2. Histomorphology of Liver*

Histomorphology of liver in *C. carpio* L. following exposure to two sublethal concentrations of glyphosate (Roundup®) *i.e.* 25% of $LC_{50}$ and 50% of $LC_{50}$ was studied at weekly intervals for a period of 28 days. The liver of control fish exhibited ordinary histoarchitecture which was characterized by normal hepatocytes containing granular cytoplasm and nuclei and presence of sinusoids (Plate II-V, Figure A).

At 7 days of exposure in $T_1$ (25% of $LC_{50}$), hepatocytes with a slight vacuolation were observed in treated group $T_1$ (Plate II, Figure B).Further, at 14 days of exposure, the structure of liver in $T_1$ (25% of $LC_{50}$), showed infiltration of leukocytes, fatty degeneration and vacuolation of hepatocytes (Plate III, Figure 6b). At 21 days of exposure, nuclear degeneration, fatty degeneration and vacuolation of hepatocytes were observed (Plate IV, Figure 8b). At 28 days of exposure too, the liver histology showed nuclear degeneration, cytoplasmic degeneration and infiltration of leukocytes (Plate V, Figure 9b).

In treated group $T_2$ (50% of $LC_{50}$), vacuolation of hepatocytes, fatty degeneration and nuclear degeneration was observed at 7 days of glyphosate exposure (Plate II, Figure 6c)and the alterations became severe with increasing exposure time and concentration. At 14 days of exposure, nuclear degeneration, fatty degenration and vacuolation of hepatocytes was recorded (Plate III, Figure 7c). Similarly, at 21 days of exposure infiltration of leukocytes, fatty degeneration and vacuolation of hepatocytes was noticed (Plate IV, Figure 8c). Further, at 28 days of exposure, infiltration of leukocytes, severe vasodilation, cytoplasmic degeneration and vacuolation of hepatocytes was observed (Plate V, Figure 9c).

The histopathological observations during the present study revealed that the alterations in the structure of liver were time and concentration dependent *i.e.* the severity of aberrations increased with increasing concentrations as well as with time of exposure. As the liver is a detoxification centre of toxicants, the hepatic alterations recommend recruitment of self-protective mechanism in an attempt to detoxify the toxicant glyphosate. Vacuolation in the liver cells of exposed fish is probably either due to fatty degeneration or disparity between the rate of formation and discharge of substance in hepatocytes. Degeneration of hepatocytes showed the evidence of excessive labour

performed by the liver of fish to detoxify the chemical toxicant from its body.

Earlier studies of Couch (1975), congestion of sinusoids occurred only due to pathological effects of organochlorine herbicides, but Neskovic*et al* (1996) also reported congestion of sinusoid, early fibrosis and lesions in the liver of *C. carpio* after exposure to glyphosate concentration (10.0mg/l). Later the findings of Jiraungkoorsul *et al* (2003), observed swelling of hepatocytes, lipidic vacuoles, pyknotic nuclei, focal necrosis and infiltration of leukocytes in *O. niloticus* following exposure to glyphosate showed a similarity to our observations.

Velisek *et al* (2014) too, observed that liver structure was changed in *C. carpio* treated with prometryne, a herbicide used to control grass in agricultural practices. Diffused steatosis (=abnormal retention of lipids within a cell) due to lipid inclusions in hepatic cells and loss of cellular shape was noticed. Similarly, slightly vacuolated cells were observed in the liver of *C. carpio* following exposure to glyphosate probably resulting from fatty degeneration. Necrosis was also noted in some portions of the liver tissues which may be due to excessive work performed by the fish to get relieved from the toxicant during the detoxification process. Inability of fish to rejuvenate new hepatocytes may be another reason for the liver necrosis (Deivasigamani 2015). Similar observation have been reported by Ayoola (2008) on glycogen vacuolation, hemosiderosis (a sign of organ dysfunction), fatty infiltration and congestion of central vein in liver of *Clarius gariepinus* after exposure to 1.9 to 9mg/l concentration of glyphosate and the severity of necrosis was recorded at 21 and 45mg/l concentration of glyphosate.

Stoyanova *et al* (2015) determined the changes induced in liver of *C. carpio* following exposure to glyphosate. The authors observed granular degeneration, balloon and fatty degeneration, necrotic alterations, lymphocyte proliferation and hyperemia in liver of exposed fish. Likewise, Stoyanova*et al* (2016) demonstrated hepatic necrotic and degenerative aberrations including granular, balloon, fatty degeneration, karyopyknosis, karyorrehsis and karyolysis in *Aristichthysnobilis* Rich. following exposure to thiamethoxam. Also, lymphocyte proliferation and hyperemia suggested abnormalities in the hepatic blood circulation.

Duarte *et al* (2008) too, recorded lipidic vacuolization and congestion in liver of *P. brachypomus* when exposed to Roundup®. Similarly, Jones *et al* (1997) noticed severe lipidic alterations in liver of fish exposed to Roundup®and attributed these alterations to changes in lipoprotein formation which is important for discharge and transport of lipids from the hepatocytes and failure in protein synthesis and transcription. Likewise, Moustafa *et al* (2016) recorded severe congestion of hepatoportal blood vessels, multifocal areas of coagulative necrosis invaded with numerous leukocytes and erythrocytes, severe hydropic degeneration and macrovesicular steatosis, periductal fibrosis, round cells infiltration besides hyalinization in the wall of blood vessels, coagulative necrosis and perivascular edema in the liver of *C. gariepinus* when exposed to Roundup® and Stomp herbicides. Samanta *et al* (2016) have also observed, vacuolation in the cytoplasm, enlarged and pyknotic hepatocytes, excess fat deposition, and inflammation of hepatocytes and enlarged acentric nuclei, vacuolation in the cytoplasm and increase in sinusoidal space in *Heteropneustes fossilis* when exposed to glyphosate-based herbicide, Excel Mera 71.

On the effect of insecticides, Devi and Mishra (2013) recorded moderate to severe alterations in liver of *C. punctatus* following exposure to Hilban® (Chlorpyrifos) which was exposure related.The major changes observed in the liver were i.e. nuclear hypertrophy, nuclear vacuolation, granular cytoplasm, bile stagnation and necrosis, dilation of lumen of sinusoid, cellular breakdown and cytoplasmic vacuolation, pyknosis, and karyokinesis.

Hundet and Prabhat (2014) reported histopathological changes in the liver of *C. carpio* following exposure to 0.0015 ppm concentration of endosulfan for period of 10 days and observed injury ofcell membrane of liver cells and connective tissues, rupture of hepatic cords, vasodilation, larger bile ductules and hepatic ducts, reduction in pancreatic tissue with indistinguishable endocrine and exocrine parts which could be consequence of pancreatic necrosis and vacuolation. At 20 days of exposure the histopathology of liver showed formation of vacuoles, complete damage of connective tissues at various places, deterioration of nucleus of hepatic cells, shrinkage of pancreatic tissues. Hypertrophy of hepatocytes, cloudy swelling, severe vacuolation of hepatocytes, hemorrhages, focal necrosis, degeneration of hepatocytes and necrosis have been reported by Monir *et al* (2015) in *Pangasianodon hypophthalmus* following exposure to cypermethrin.


***Figure 5a.*** *Cyprinus carpio L. ($T_1$) showing loss of equilibrium.*


***Figure 5b.*** *Cyprinus carpio L. ($T_2$) showing erratic swimming.*

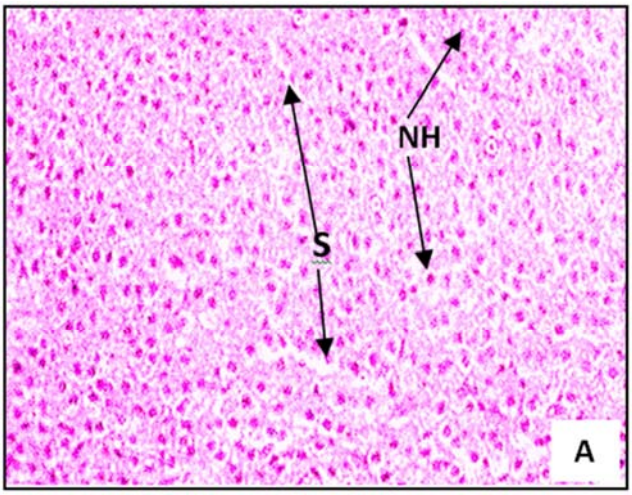


***Figure 6a.*** *T. S of liver (control) showing normal hepatocytes (NH) and sinusoids (S) (40X).*

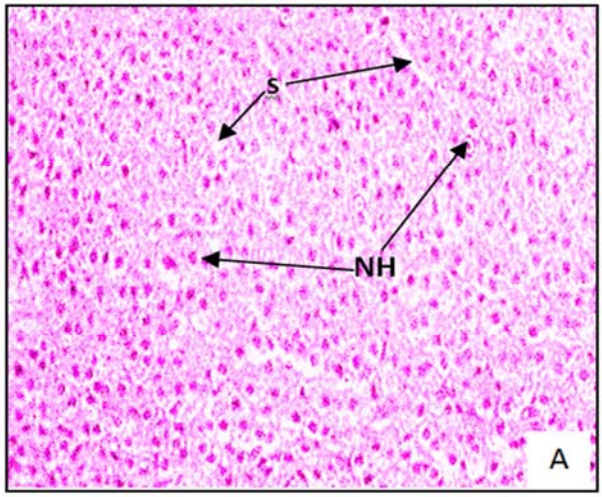


***Figure 7a.*** *T. S of liver (control) showing normal hepatocytes (NH) and sinusoids (S) (40x).*

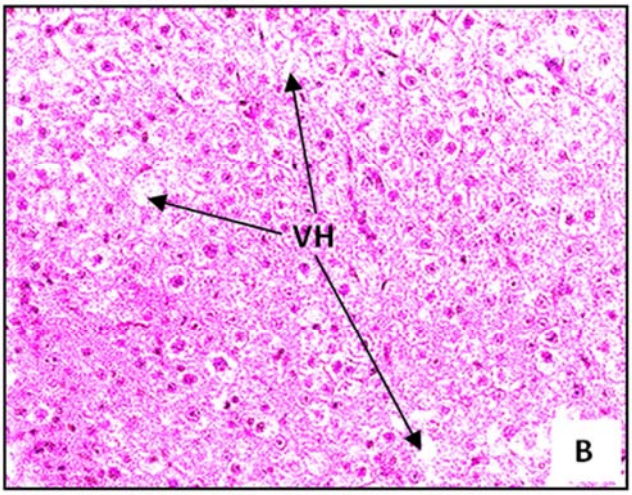


***Figure 6b.*** *T. S of liver ($T_1$) after 7 days of exposure to glyphosate showing slight vacuolation of hepatocytes (VH) (40X).*

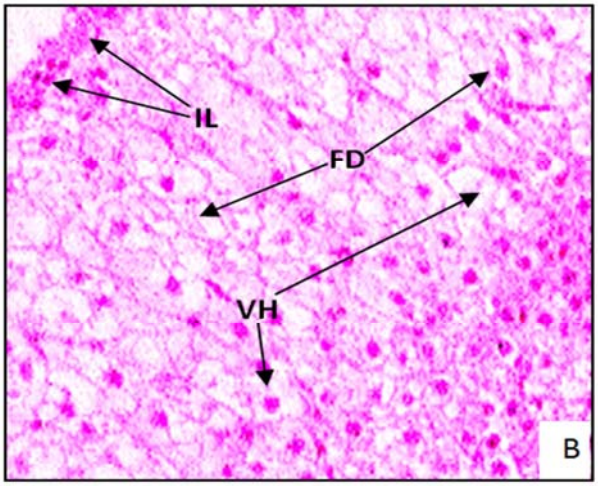


***Figure 7b.*** *T. S of liver ($T_1$) after 14 days of exposure showing infiltration of leukocytes (IL), fatty degenration (FD) and vacuolation of hepatocytes (VH).*

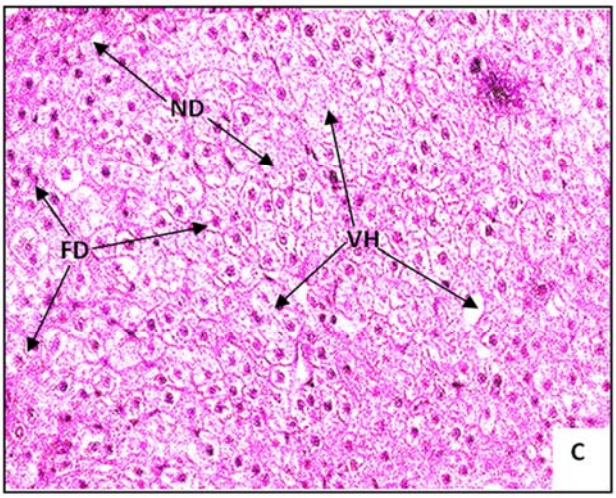


***Figure 6c.*** *T. S of liver ($T_2$) after 7 days of exposure to glyphosate showing vacuolation of hepatocytes (VH), fatty degenration (FD) and nuclear degeneration (ND) (40X).*

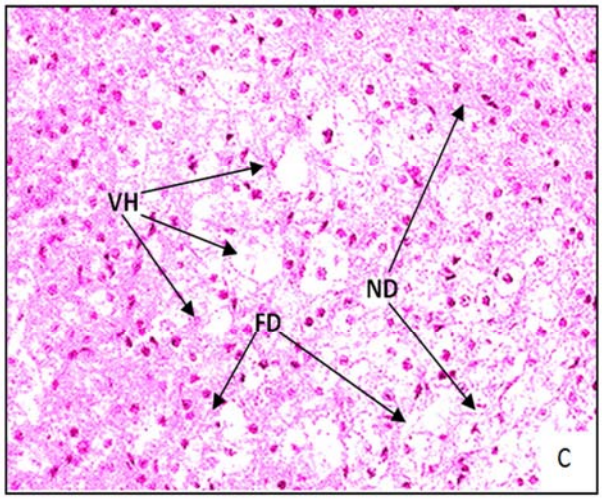


***Figure 7c.*** *T. S of liver ($T_2$) after 14 days of exposure showing nuclear degeneration (ND), fatty degenration (FD) and vacuolation of hepatocytes (VH) (40x).*

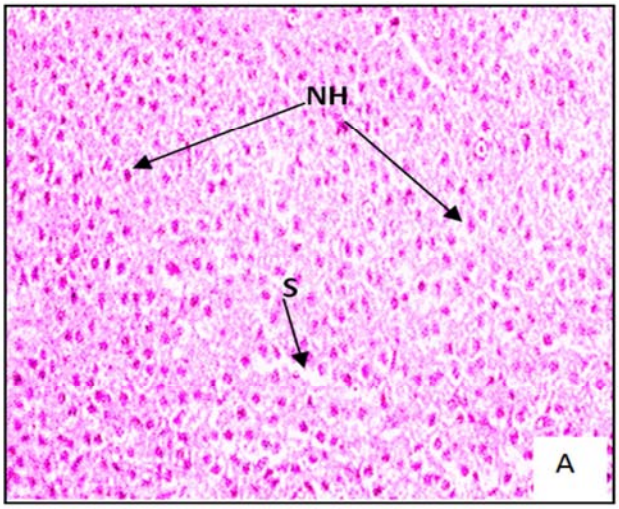


***Figure 8a.*** *T. S of liver (control) showing normal hepatocytes (NH), sinusoids (S)(40X).*

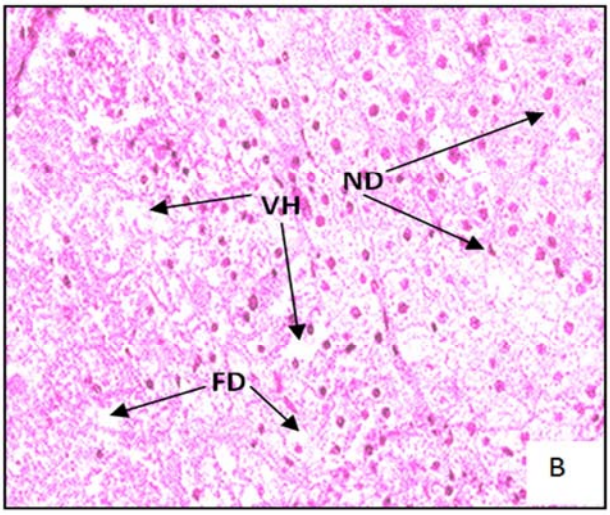


***Figure 8b.*** *T. S of liver ($T_1$) after 21 days of exposure showing nucleardegeneration (ND), fatty degeneration (FD) and vacuolation of hepatocytes (VH)(40X).*

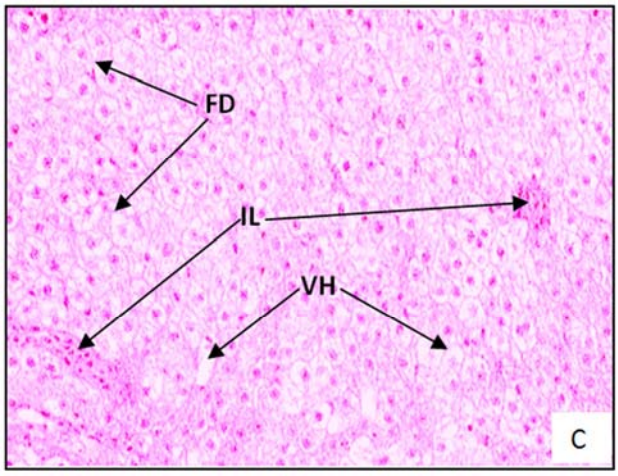


***Figure 8C.*** *T. S of liver ($T_2$) after 21 days of exposure showing infiltration ofleukocytes (IL), fatty degeneration (FD) and vacuolation of hepatocytes (VH)(40X).*

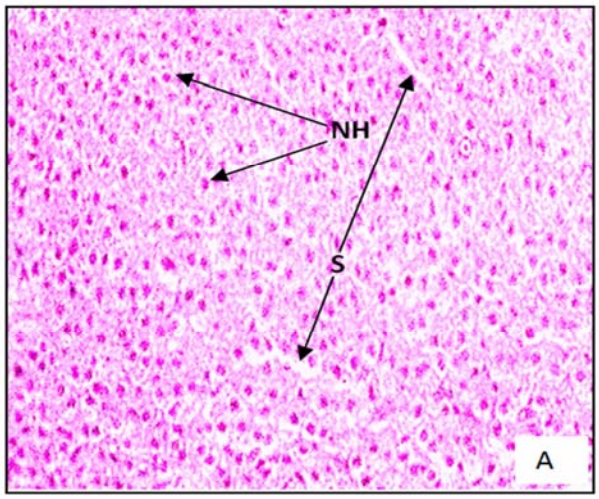


***Figure 9a.*** *T. S of liver (control) showing normal hepatocytes (NH), sinusoids (S) (40X).*

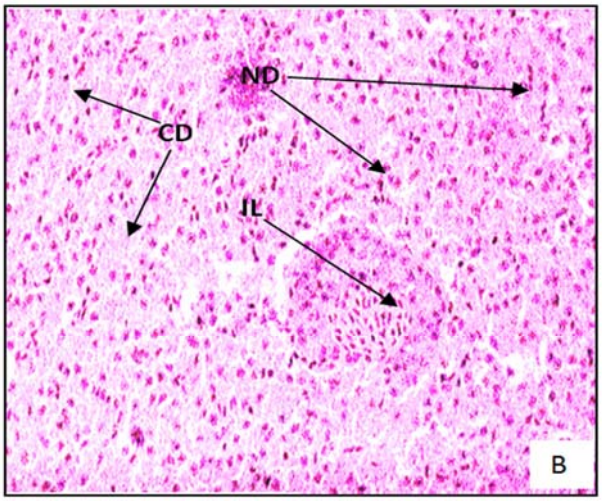


***Figure 9b.*** *T. S of liver ($T_1$) after 28 days of exposure to glyphosate showing nuclear degeneration (ND), cytoplasmic degeneration (CD) and of leukocytes (IL)(40X).*

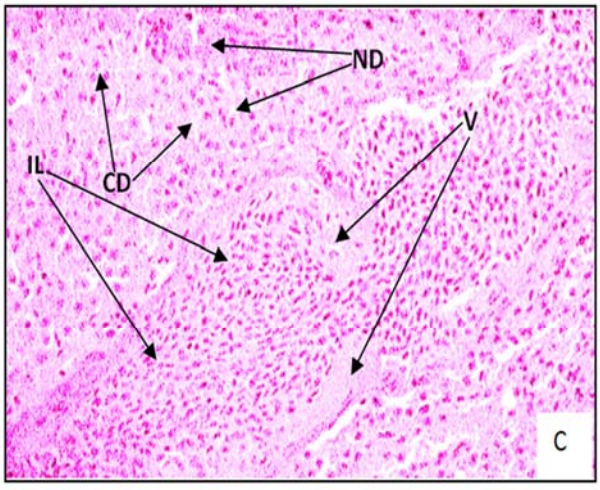


***Figure 9c.*** *T. S of liver ($T_2$) after 28 days of exposure to glyphosate showing infiltration of leukocytes (IL), vasodilation (V), cytoplasmic degeneration (CD) and vacuolation of hepatocytes (VH)(40X).*

# 4. Conclusion

The present study concluded that chronic exposure to glyphosate induces biochemical and histopathological alterations in the liver of *C. carpio*. Glyphosate (Roundup®) caused decrease in protein and lipid content in the liver of *C. carpio* and an increase in the activities of AST and ALT (aminotransferases). Glyphosate (Roundup®) also induces marked aberrations in histoarchitecture of the liver of the exposed fish. These are sensitive biomarkers of xenobiotic exposure and also pointers to the huge consequences to our health because glyphosate was originally patented as a mineral chelator, whereby it immobilizes nutrients, making them unavailable in the body, possibly leading to improper metabolism caused by molecular mimicry.

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