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# Ecotoxicological Impacts of Roundup (glyphosate 41%) in Freshwater Fish Grass Carp *Ctenopharyngodon idella*: Hematological, Biochemical and Enzymological Response

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**ABSTRACT: The chronic and sublethal effects of herbicide were anticipated to have an effect on the serum biochemical, metabolic, and enzymatical characteristics of a freshwater fish known as a grass carp *Ctenopharyngodon idella*. The median lethal concentration of herbicide in fish grass carp for 96 hours was found to be 0.15 mg/L. The levels of hemoglobin (Hb), hematocrit (Hct), red blood cells (RBC), white blood cells (WBC), plasma glucose, plasma protein, liver aspartate (AST) and alanine aminotransferase (ALT), and others all lowered during acute treatment (0.15 mg/l), whereas corpuscular indices like mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) enhanced in fish exposed to herbicide likewise MCHC and blood glucose levels rose over course of effects Hb, RBC, and plasma protein levels decreased in the sublethal dose (0.15 mg/l) of glyphosate. WBC, MCH, liver AST, and ALT values all had a biphasic trend. It is possible to swiftly and effectively determine the health of fish exposed to glyphosate in their aquatic habitat by altering.**

**Keywords:** Glyphosate, Grass carp *Ctenopharyngodon idella*, Hematological, Biochemical, Enzymological, Aspartate amino transferase (AST), Alanine amino transferase (ALT).

## INTRODUCTION

Water contamination has become a major concern in this century due to the introduction of numerous pollutants that alter the natural properties of water (Voltz *et al.,* 2005). These pollutants have harmful effects on non-target organisms and can cause death after both short-term and long-term exposed (Velcheva *et al.,* 2012; Sabae *et al.*, 2014). Pesticides, which are a major source of environmental pollution, contaminate soil and other components of aquatic and terrestrial habitats where they are used (Lytle and Lytle 2001). Glyphosate- formed herbicides are frequently utilized globally and dominateherbicide market in Brazil (IBAMA, 2010). One of the most frequently used herbicide formulations is the broad-spectrum Roundup® (Fan *et al.,* 2013), which has become more prevalent due to the widespread use of genetically engineered seeds that are resistant to Roundup (N-(Phosphonomethyl) glycine) (Pengue, 2017; Santadino *et al.,* 2014). A commercial version of glyphosate, known as Roundup (glyphosate 48%), is made using isopropyl amine salt, which is the acidic counterpart of the Roundup herbicide (Caballero-Gallardo *et al.,* 2016). To enhance its effectiveness, surfactants such as polyethoxylated tallow amine (POEA) are often added (De Moura *et al.*, 2017). Both the active element (glyphosate) and the manufactured product (Roundup) are equally hazardous. Numerous studies have shown that Roundup causes cellular oxidative stress by producing reactive oxygen species (ROS) and/or disrupting the antioxidant system (Webster and Santos 2015). The aquatic environment is known to be vulnerable to glyphosate-based herbicides as they are harmful to aquatic species and have long-lasting effecting (Sihtmae *et al.*, 2013). A wide range of fertilizers, including pesticides, fungicides, insecticides, and herbicides, are used in modern agriculture. Herbicides make up more than 50% of all pesticides used globally and have seen rapid growth in pesticide industry (Dumontet *et al.,* 2012). They applied to remove weeds, trees, and bushes along highways and to control aquatic vegetation in water bodies such as ponds and lakes. Herbicides can work in various ways, such as controlling growth, inhibiting seedling growth, or inhibiting photosynthesis, depending on their nature. Some herbicides can affect metabolism by hindering the production of lipids and amino acids or damaging cell membranes (Lushchak *et al.*, 2018).

Herbicides can enter freshwater systems through runoff, water, or spray drift and harm non-target species like aquatic invertebrates, impairing the environment's ability to function. This has been documented by Schaaf (2017); Vera *et al.* (2012). Herbicides can persist in aquatic habitats for an extended period of time after being introduced through runoff and leaching from agricultural practices (Mai *et al.,* 2013). Fish play a crucial role in assessing the potential risks posed by pollutants in freshwater aquatic environments and serve as biological indicators of environmental pollution (Lakra and Nagpure 2009). The use of fish as a biological monitoring tool for

evaluating the impacts of pollution is becoming increasingly important, as it allows for early detection of problems in aquatic environments (Van Der Oost *et al*., 2003). Herbicide effects on gonads, fertilization, and cell proliferation are well understood at various concentrations. However, research on acute and chronic herbicide toxicity has shown that consumers have an impact on the longevity of freshwater organisms (Llanos-Rivera et al., 2009). To study the effects of atmospheric toxins, it is important to assess their impact on key organisms. A comprehensive evaluation using indicators across various stages of biological structure should be conducted to fully understand the negative effects (Bonifacio *et al.,* 2016). Hematological and biochemical factors, as well as ion regulation and enzymological markers, are frequently used as biological indicators to evaluate the contamination of environmental pollutants in an ecosystem (Velisek *et al.,* 2010). Hematological factors such as red blood cell (RBC), white blood cell (WBC), and hemoglobin, as well as biochemical indices like cellular hemoglobin concentration (MCHC) and mean cellular hemoglobin (MCH) plasma glucose and protein, are commonly used to measure toxic stress (Llanos-Rivera *et al.,* 2009). Alterations in enzyme activity can also be used to identify cell damage and abnormalities in creatures exposed to long-term toxicant dosages (Ozmen *et al.,* 2006). The presence of toxic substances in aquatic environments can lead to tissue damage in aquatic organisms, and this can be evaluated by measuring the activities of transaminases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Nemcsok and Benedecky 1990). AST facilitates the conversion of the chemical group from aspartic acid to α-ketoglutaric acid, while ALT catalysisa conversion of amino group from alanine to α-ketoglutaric acid, resulting in the formation of pyruvic acid and glutamic acid. These enzymes play an important role in the relationship between protein and glucose synthesis (Harper *et al.,* 1978) and are necessary for using nucleotide bases for carbohydrate metabolism or oxidative stress (Rodwell, 1988).

The liver of fish species has been found to be a potential indicator of the negative impacts of pollution. The early life stages of these species are particularly susceptible to water pollutants. A study by Jiraungkoorskul *et al.* (2003) suggests that some biochemical indicators in the fish liver are effective in detecting these effects. Furthermore, liver micro histology has been proven to be a significant biological indicator of hazardous impacts as changes in cellular and sub-cellular structures can be used to predict stress in isolated organisms from chronic or long-term exposure to pollutants (Stentiford *et al.,* 2003). The liver, being a target organ impacted by toxicant exposure and being the major organ for xenobiotic metabolism in fish (Mohamed, 2009) was used in a study to assess the chronic and sub-chronic toxic exposure of the herbicide on the grass carp *Ctenopharyngodon idella*, through examination of biochemical, hematological, and enzymological data.

## MATERIAL AND METHODS

*A. Glyphosate (Roundup)*

The active ingredients of Roundup, Glyphosate 41% and Surfactant POEA, were purchased from Monsanto India Private Limited in India. The Roundup stock solution was then diluted in distilled water to form a final concentration of 1 litre.

*B. Animal maintenance*

In a 1000-liter capacity cement tank, grass carp *Ctenopharyngodon idella* specimens were integrated for 3 weeks under controlled laboratory circumstances. The fish were sourced from the Siraco fish farm in Nerunjipettai, erode district, Tamilnadu, India, and measured 7.0 ± 0.5 cm and weighed 10.5 ± 1.2 g. During the acclimation phase, the fish were given free access to rice flour, and groundnut oil cake, and the water was replaced daily to removed excretory debris. 24 hours passed between feedings and the start of the trial. The water used in the trial was unchlorinated tap water with the following characteristics: temperature of 26.0 ± 1.2 ˚C, pH of 7.1 ± 0.02, dissolved oxygen of 6.5 ± 0.05 $mgl^{-1}$, salinity of 0.5 ± 0.02 ppt, alkalinity of 15 ± 8.5 $mgl^{-1}$, calcium of 4.5 ± 0.2 $mgl^{-1}$, total hardness of 12.5 ± 0.5 $mgl^{-1}$, and magnesium of 3.5 ± 0.2 $mgl^{-1}$, determined by the APHA (1998) method.

*C. $LC_{50}$ value*

One gram of glyphosate was solubilized in an appropriate volume of tap water to create a stock solution. Ten fish were placed in each of several 50-liter glass aquariums, and were randomly selected from the stock. The fish were treated with various concentrations of the herbicide (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ppm) in order to determine the median lethal concentration ($LC_{50}$) of the herbicide. Fresh water mixed with the herbicide was added daily to keep the consistently concentration of the chemical throughout the exposure period. The treated was conducted with three multiplies at each concentration, and control aquariums were maintained under the same conditions. After 96 hours, fish mortality was measured and the $LC_{50}$, which was 0.15 ppm, was determined as the concentration at which 50% of the fish died (Finney 1978). The sublethal concentration for research was found to be $1/10^{th}$ of the $LC_{50}$ value for 96 hours, or 0.15 ppm (Sprague 1971).

*D. Acute test for 96 hours*

For the acute toxicity test, three plastic containers with a combined volume of 50 litres and 40 litres of water were filled with 60 fingerlings, with 20 fish in each group. Glyphosate amount of 0.15 mg/L, equal to the 96-hour LC50 value, was added to each container. At the same time, three control containers were also maintained. After 96 hours, live fish from the glyphosate-treated containers and the control container were randomly selected for serum was accumulated for hematological and biochemical examination. Simultaneously, muscle and liver samples were taken to estimate glycogen.

*E. Sublethal exposure*

A sample of 300 grass carp was selected randomly from the stock, and 100 fish from each aquarium were separated into three groups, with one group serving as the control and two serving as the experimental groups. The experiment was conducted over a 21-day period, with measurements taken at 7-day intervals. To keep the toxicant's test contains, 0.15 mg/l of glyphosate, $1/10^{th}$ of the 96-hour $LC_{50}$ value, was added to two test aquariums and refilled daily after removing a similar amount of water. No fish deaths were recorded during the entire experimental period.

*F. Blood sample collection*

Using a heart puncture, blood serum was collected from both the glyphosate-experimental and treated groups with a disposable syringe and a 26-gauge needle that was pre-moistened with anticoagulation of heparin. The collection blood was immediately placed on ice in plastic vials that contained heparin. Entire blood was used to estimate the Hb, RBC, and WBC counts, and the plasma was separated through a 20-minute centrifugation process at 10,000 rpm to determine the glucose and protein levels.

*G. Estimation of Hematological profile*

The RBC and WBC were estimated in million/cu mm and 1000/cu mm, respectively, refer to the methodology of Rusia and Sood (1992). The hemoglobin content of the blood serum was calculated in g/dl using the Drabkin (1946) method. The hematocrit, which represents the proportion of the volume of packed red blood cells to the total blood volume, was calculated and expressed as a ratio according to Nelson and Morris (1989). The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), which are indicators related to erythrocytes, were determined and represented in g/dl, respectively.

*H. Biochemical Test*

(i) **Determination of plasma glucose.** To determine the plasma glucose levels, the Cooper and McDaniel technique (1970) was employed. 0.1 ml of bloodserum was added to 5 ml of O-toluidine colour. The mixture was thoroughly mixed and then heated in boiling water for 10 minutes. During 30 min, the experimental sample's optical density (OD) was determined at 630 nm in a UV spectrophotometer and reported as mg/100 ml. After that, the sample was cooled for 5 minutes under running water.

(ii) **Determination of plasma protein.** The blood protein levels were estimated using the Lowry et al. (1951) technique. The procedure involved mixing 0.10 mL of plasma with 0.90 mL of distilled water and then treating the mixture with a solution of sodium carbonate and copper sulphate. After standing for five minutes, the colour intensity was measured using a UV spectrophotometer and represented in g/ml.

*I. Determination of Enzyme*

(i) **Enzyme assay**. While collecting blood via the grass carp, they were thoroughly dissolved with double-distilled water and dried using absorbent sheet. The fish were then divided into control and experimental groups, and their livers were homogenized under cold conditions with a 2.5 mL solution of 0.25 M sucrose, using 100 mg of each tissue (Hogeboom *et al.,* 1948). The homogenized mixture was then centrifugation for 20 minutes at 6000 rpm to collect the transparent supernatant solution, which was used enzyme testing.

(ii) **AST activity.** The Reitman and Franckel technique (1957) were used to measure the activity of AST in the liver. 50 ml of the hepatocyte's supernatant were mixed with 0.25 ml of buffer aspartate. The combination was then kept for a further 60 minutes at 37 °C before 0.25 ml of 2, 4-DNPH was added and an additional 20 min at room temperature were spent incubating the mixture. After adding 2.5 ml of 0.4 N NaOH to the mixture, it was let to stand for 10 minutes. A UV Spectrophotometer was used to measure and quantify the optical density (OD) at 505 nm. The enzyme activity was calculated using the standard curve and represented as IU/L simultaneously with a simultaneous study of the standard graph.

(iii) **ALT activity.** The ALT activity in the liver was measured using Reitman and Franckel's technique (1957). The procedure involved adding 0.25 ml of buffer Lalanine to 50 ml of the liver homogenate supernatant. The mixture was then incubated at 37°C for 30 minutes. After being mixed with 0.25 ml of 2,4-DNPH, the mixture was left to stand for 20 minutes at room temperature. The mixture was then thoroughly blended with 2.5 ml of 0.4 N NaOH and left to stand for 10 minutes. The optical density was determined using a UV Spectrophotometer at 505 nm. A concurrent standard curve analysis was also performed, and the enzyme activity was reported as IU/L, which was interpreted using the standard curve.

*J. Statistical analysis*

The data was analyzed statistically using a p-value of less than 0.05. The significance of the difference between the experimental and control data was determined using a student's t-test, which generated the t values.

Result

(i) **LC50 Value for 96 h.** In this study, the LC50 96-h value of glyphosate for grass carp was determined and found to be 0.15 mg/l.

(ii) **Chronic toxicity test.** In the current investigation, the fish *Ctenopharyngodon idella* treated with glyphosate showed a significant decrease ($p < 0.05$) in the levels of hematological parameters, such as hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC), as well as biochemical parameters, such as plasma glucose and protein, and enzymatic parameters, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), compared to the fish in the control group, as shown in Table 1. However, in the fish treated with glyphosate, there was also a substantial decrease ($p<0.05$) in other hematological indices, such as mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), as indicated in Table 1.

*K. Sublethal toxicity test*

(i) **Hematology test.** Throughout the exposure period, fish *Ctenopharyngodon idella* treated with glyphosate

showed a decline in hematological parameters such as Hb, RBC, and WBC concentration ($p<0.05$), with the largest decrease occurring end of the 21st day (Fig. 1a-c). MCH concentration also significantly reduced ($p<0.05$) until the 14th day (Fig. 1d) before continuing to decrease throughout the experimental duration. Furthermore, a slight reduced in MCHC concentration was observed in glyphosate-experimental fish compared to the control group throughout the study (Fig. 1e).

(ii) **Biochemical analysis.** The plasma glucose levels of the fish *Ctenopharyngodon idella* treated with glyphosate were significantly higher ($p<0.05$) compared to their respective control fish (Fig. 2a). Additionally, the plasma protein levels of the glyphosate-treated fish were significantly lower ($p<0.05$) throughout the experimental period, and this decrease correlated directly with the length of exposure time (Fig. 2b).

(iii) **Enzyme analysis.** The liver AST activity in fish *Ctenopharyngodon idella* treated with herbicide initially increased up to day seven, but then showed a substantial reduced ($p<0.05$) comparison of the control group after the 14th day, as indicated in Figure 3a. Similarly, there was a small decrease in ALT activity by day seven, followed by a significant ($p<0.05$) decrease for the remainder of the study period, as shown in Fig. 3b.

**Table 1: Exposure of hematological, biochemical and enzymological parameters in a freshwater fish grass carp *Ctenopharyngodon idella* during acute treatment of glyphosate.**

| Parameters | Control | Experiment | percentage |
|---|---|---|---|
| **Hematological Parameters** | | | |
| Hb(g/dl) | 8.72 ±0.020 | 5.63 ±0.027** | -3.54 |
| RBC (million /cu mm) | 6.84±0.011 | 4.83±0.031** | -3.68 |
| WBC (1000/cu mm) | 5.97±0.029 | 3.47±0.04** | -4.18 |
| MCH (picograms) | 28.87±0.026 | 24.64±0.04** | -14.65 |
| MCHC (g/dl) | 3.85±0.023 | 2.44±0.02** | -3.66 |
| **Biochemical Parameters** | | | |
| Plasma glucose (mg/100ml) | 106±2.068 | 85±2.54* | +198.11 |
| Plasma protein (µg/ml) | 7.8±0.175 | 3.7±0.091** | +52.56 |
| **Enzymological Parameters** | | | |
| Liver AST (IU/L) | 87±1.31 | 66±1.636* | -24.18 |
| Liver ALT (IU/L) | 95±0.8843 | 58±0.9* | -38.94 |

Value is the average of five separate observations;
(+) indicates a percentage increase compared to the control, and (-) indicates a percentage drop compared to the control.
* Values are significant at $p < 0.05$.

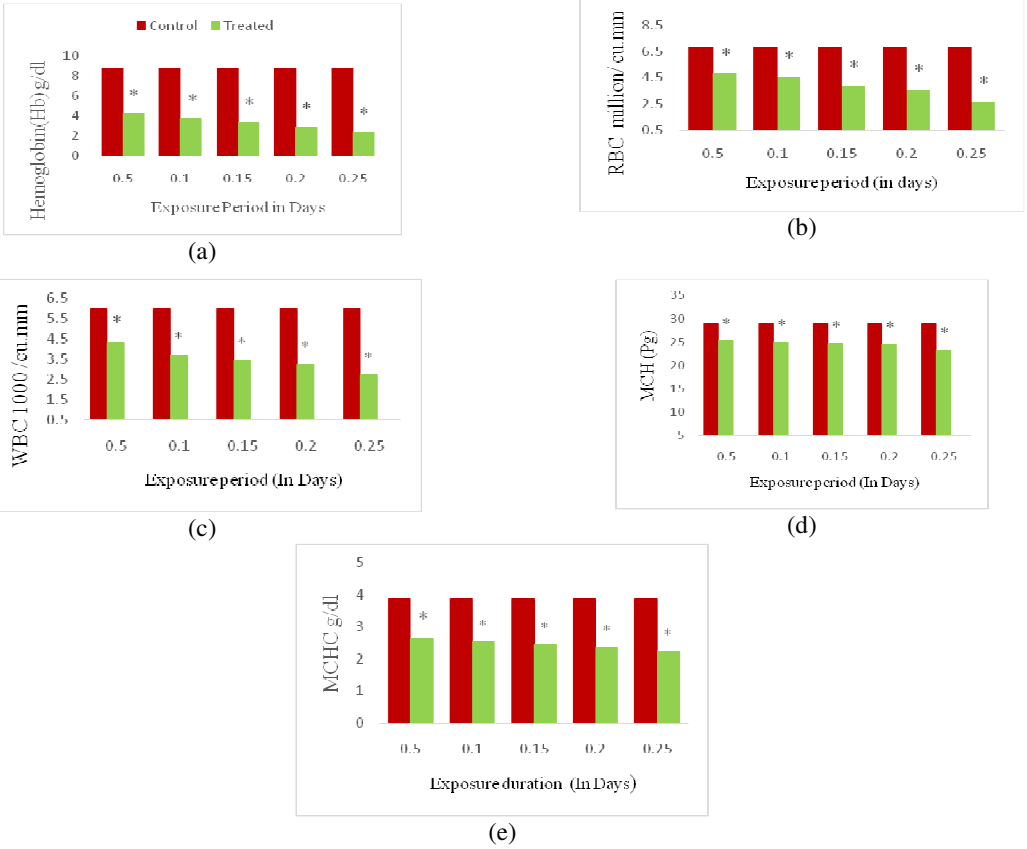
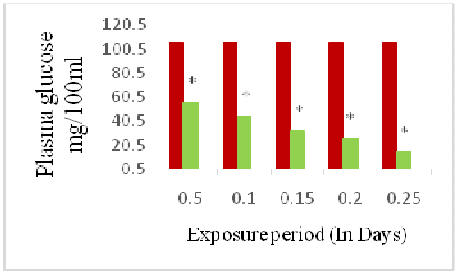
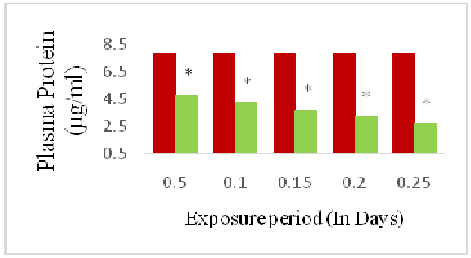



**Fig. 1.** Hematology concentration (a. Hb; b. RBC; c. WBC, d. MCH; e. MCHC) of *Ctenopharyngodon idella* exposure to glyphosate for 21 days. Bar graph represents SE of the mean. Using a student t-test, the means of control and treated fish were compared. * At a 5% level, significant ($p<0.05$).

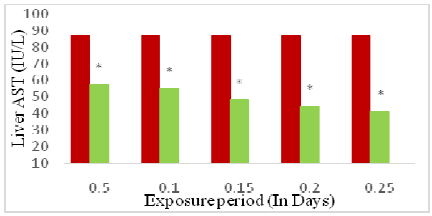



(a)

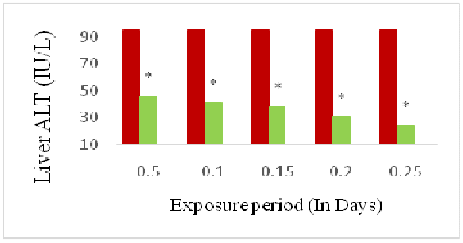



(b)

**Fig. 2.** Biochemical changes in Plasma protein and glucose levels following a 21-day glyphosate treatment in *Ctenopharyngodon idella.* The S.D of the mean is shown by a bar. Student t-tests were used to compare the means of control and treated fish. * Significant at the 5% level ($p < 0.05$).




(a)




(b)

**Fig. 3.** Enzymatical change (a. AST; b. ALT) of *Ctenopharyngodon idella* effected to glyphosate for 21 days. Bar graph represents SE of the mean. The student's t-test was used to compare the means of the control fish and the treated fish. ($p<0.05$) Significant at the 5% level.

## DISCUSSION

Previous studies have explored the potential negative effects of glyphosate exposure on the development of various fish species and aquatic organisms (Li *et al.,* 2017; Lopes *et al.,* 2017; Sobjak *et al.,* 2017; Stehr *et al.,* 2009; Sulukan *et al.,* 2017; Amid *et al.,* 2017; Mottier *et al.,* 2013; Schaumburg *et al.,* 2016; Wagner *et al.,* 2015). The LC50-96h value of Roundup was determined to be 22.19 ppm in the current investigation (Neskovic, 1996). The LC50-96h value for grass carp exposed to glyphosate was found to be 620 ppm, which is higher than the values obtained for other species such as *Oreochromis niloticus* (16.8 ppm), *Gambusia yucatana* (17.8 ppm), *Leporinus macrocephalus* (15.2 ppm), and *Prochilodus lineatus* (13.7 ppm) (Jiraungkoorskul *et al.,* 2002; Osten *et al.,* 2005; Albinati *et al*., 2007; Langiano and Martinez 2008). In another study, the LC50-48h value for *Piaractus mesopotamicus* treated with Roundup was found to be 3.74 ppm (Peixoto, 2005). The variance in the toxicity of glyphosate formulations may be due to differences in surfactant chemicals such as POEA, as well as factors like fish species, water hardness, pH, and salinity (Cattaneo *et al.,* 2011). It should be noted that glyphosate alone is considered to be less toxic to fish than the surfactant POEA (Giesy *et al.,* 2000). Toxicity tests using glyphosate and Roundup® have been frequently conducted to assess the impact of these substances on fish species in terms of physiology, biochemistry, and genotoxicity (Glusczak *et al*., 2006; Langiano and Martinez 2008; Modesto and Martinez 2010a, b; Glusczak *et al.,* 2007; Cavalcante *et al.,* 2008; Guilherme *et al.,* 2012b; Moreno *et al.,* 2014). However, exposure to herbicides at concentrations ranging from 0.1 to 100 mg/l did not have a significant effect on the survival, weight, length, or condition of larvae. These results align with previous studies (Mitchell *et al.,* 1987; Morgan and Kiceniuk 1992; Huang *et al.,* 2005). To the best of our knowledge, the effect of glyphosate exposure on common carp during early life stages has not been studied. Our study evaluates the toxic impact of glyphosate on both fish species, *D. rerio* and *C. carpio*, utilizing endpoints such as hatching rate, mortality, and deformities. Many studies have reported increased mortality in young fish due to Roundup and glyphosate-based herbicides (Zhang *et al*., 2017). For instance, Yusof *et al.* (2014) found in only 50% of Java medaka oocytes survived after exposure to 100 mg/l of glyphosate for 16 days. Langiano and Martinez (2008) also mentioned an enhanced in plasma glucose levels in streaking prochilod after treatment with 10 mg/l of Roundup for 96 hours. Fish exposed to Roundup have also shown hematological and hematopoietic changes, but most of these changes are not associated to herbicide's concentration. Compared to the control group, fish effected to Roundup exhibited a reduce in hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC). The limited research on the hematological effects of glyphosate-based herbicides in fish indicates that exposed fish generally have lower red blood cell (RBC) parameters, suggesting anaemic reactions. Gholami-Seyedkolaei *et al.* (2013) found that sublethal doses of Roundup (3.5, 7, or 14 mg/l) significantly reduced Hb, hematocrit (Hct), and RBC levels in common carp (*Cyprinus carpio* L) after 16 days. Meanwhile, MCV and MCH values increased and MCHC values decreased. Orun *et al.* (2013) reported that Hb, RBC, Hct, and MCV values in juveniles common carp treatment to 6 and 12 mg/l of glyphosate

for 4 and 21 days similarly declined. Similarly, Salbego *et al.* (2010) found that Piava (*Leporinus obtusidens*) treated with 1 or 5 mg/L of Roundup for 90 days exhibited a reduced in Hct, Hb, and RBC concentration. Glusczak *et al*. (2006) reported that Piava exposed to glyphosate (3–20 mg/l) showed decreased Hct, Hb, and RBC values than control group. Svobodova *et al.* (1997) attributed the reduced in Hb and Hct levels in fish exposure to Roundup to abnormalities in hematopoietic techniques and rapid breakdown of the red blood cell layer. Fish exposed to herbicides show signs of hemolysis, hemorrhage, and impaired erythropoiesis, which can be related to the decrease in Hb (Khan *et al.,* 2016). According to Kreutz *et al.* (2010), the silver catfish exposed to roundup had lower WBC levels, which could explain why there were fewer cells in the coelom cavity. Herbicide toxicity in renal tissue, which is the centre of activity of hematopoiesis, may be another reason for the decline in WBC (Kotsanis *et al*., 2000). In our investigation, the percentages of the majority of hematopoietic cell division in fish exposed to Roundup did not alter considerably; nevertheless, the volume of monocytic, eosinophilic, and basophilic lineage cells increased dramatically. Additionally, fish exposure to Roundup at all concentrations showed a considerable rise in the percentage of lymphocytes. In this investigation, exposure to sublethal concentrations of Roundups resulted in a statistically significantly reduce ($p< 0.05$) in the WBC count and percentage of lymphocytes of *C. carpio* obtained significant results Kreutz *et al.* (2011).

Typically, the presence of contaminants in aquatic environments affects cellular and molecular processes, leading to significant changes in biochemical reactions (Vutukuru, 2003). Biochemical analysis is an important tool for monitoring aquatic environments and detecting environmental stress in fish. Plasma glucose is commonly used as a sensitive indicator of stress in fish (Nemcsok and Boross 1982). In this study, an increase in plasma glucose levels was observed in fish subjected to acute and sublethal treatments with insecticides. This increase is likely due to the stimulation of gluconeogenesis, which produces glucose to meet increased metabolic demands caused by the stress of the insecticide. Furthermore, stress triggers the hypothalamus-pituitary-interrenal axis, increasing cortisol levels and leading to lipolysis, glycogenolysis, and gluconeogenesis, providing energy in response to stress (Sheridan, 1986; Hontela, 1993).

To better understand the health and metabolic mechanisms of fish under environmental pollution and stress, protein levels are also a crucial biochemical marker. During stressful situations, fish may mobilize protein to meet increased energy needs. (Martinez 2004). The amount of glycogen in the liver and muscle of fish can help determine the health of the fish, as glycogen serves as the primary energy reserve during normal metabolism. Environmental stress, such as exposure to chemicals, can disrupt the fish's natural metabolism, leading to a reduction in glycogen levels. This reduction indicates that the glycogen stores are being depleted to meet the increased energy demands brought on by the stress. Studies have shown similar reductions in glycogen levels in fish exposed to dimethoate and rogor herbicides (Begum and Vijayaraghavan 1996). On the other hand, exposure to antibiotic residues can lead to a slower glycogenolysis, as seen by an increase in liver glycogen levels (Sastry, 1984). Overall, a decrease in glycogen levels could be an early indicator of regulatory changes in intermediate metabolism during stress (Kumari, 2010).

Measuring multiple enzymes can improve the accuracy of enzyme analysis, as stated by Crook (2006). The study measured four enzymes: alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase, which are present in high concentrations in the liver. Exposure to sublethal amounts of a substitute for five weeks increased the levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in Labeorohita, according to Vasanth *et al.* (2012). Abnormal liver cells can secrete specific enzymes into the bloodstream, increasing their levels, as shown in studies by Burtis and Ashwood (1999); Vutukuru *et al*. (2007). Fish exposed to contaminated water may exhibit a significant increase in enzyme activity, such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, which may be caused by impair liver cells that results an increased leakage of enzymatic into the bloodstream (Sunmonu *et al.,* 2009). Enzymes such as ALT, AST, LDH, and ALP are widely used as biomarkers to evaluate the effects of pollution on animals and water contamination. Hence, these enzymes are considered to be the most accurate biomarkers for identifying stress in fish exposed to various water contaminants (Adhikari *et al.,* 2004). In the current investigation, sublethal treatment resulted in a biphasic response of AST and ALT activity. Long-term exposure raised the activity of enzymes (AST and ALT), indicating that the organism tries to reduce the harmful and stress-induced stress by increasing rate of metabolism with prolonged exposure time. It's possible that injured hepatocytes are unable to produce AST protein, which would explain the acute and sublethal treatment-induced considerable drop in AST and ALT activity. Also, renal insufficiency may have contributed to the considerable decline in ALT activity.

## CONCLUSIONS

The results of the current study indicate that exposure to glyphosate significantly changes the hematological, biochemical, and enzymatic characteristics of grass carp *Ctenopharyngodon idella* in freshwater fish under both acute and sublethal conditions. The presence of this amount of glyphosate in a healthy environment will have a negative effect on the *viability* of fish. In findings provide a well *comprehension* of the toxic effects of this toxin and it suggest safe glyphosate concentrations for freshwater ecosystems and the preservation of aquatic life. As fish is a crucial source of protein for people worldwide, it is crucial to prevent the release of glyphosate into nearby bodies of water.

## FUTURE SCOPE

Glyphosate affected in grass carp hematological, biochemical and enzymological in the present research may be further to affects for fish hormonal effects and DNA damages.

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**Conflict of Interest.** None.

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