Biochemical Alterations Induced by Glyphosate in some Vital Organs of *Clarias batrachus* (Linnaeus, 1758)

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ABSTRACT

Glyphosate ($C_3H_8NO_5P$) is a widely used herbicide in agriculture for the control of weeds. The present study investigated the biochemical alterations induced by sublethal exposure (2.46 mg/L) of glyphosate in the air-breathing fish *Clarias batrachus* (Linn.) over a period of 30 days. Biochemical parameters such as glycogen, total protein, and total lipid were analysed in the liver, kidney, muscle, and gill tissues of treated fish. The results revealed significant reductions in all biochemical constituents compared to the control group. The percentage decrease in glycogen content was observed in the following order: kidney (−42.91%) > liver (−36.08%) > gill (−32.52%) > muscle (−26.47%). Similarly, total protein content decreased in the order: kidney (−44.19%) > liver (−34.54%) > gill (−29.30%) > muscle (−20.04%). The reduction in total lipid content was highest in muscle (−45.92%), followed by kidney (−38.07%), liver (−35.89%), and gill (−31.53%). These findings indicate severe metabolic dysfunction in response to glyphosate toxicity. Therefore, water contamination with glyphosate at concentrations ≥ 2.46 mg/L in fish culture ponds may not be suitable for optimal growth and physiological performance of *Clarias batrachus*.

Key words : *Clarias batrachus*, Herbicide, glyphosate, biochemical changes.

INTRODUCTION :

Water pollution resulting from agricultural waste discharge has become one of the most significant environmental problems worldwide (Urban, 1986). Pesticides applied in agricultural fields are often washed away by rainfall and enter nearby aquatic ecosystems, where they adversely affect non-target organisms, particularly fish. Among agricultural contaminants, pesticides represent a major class of toxicants in aquatic environments, drawing considerable attention from researchers globally (Ajani and Awogbade, 2012). Fish are especially susceptible to these pollutants due to their direct exposure to contaminated water and their role in aquatic food chains (Wekler, 2000).

Glyphosate (N-phosphonomethyl glycine; $C_3H_8NO_5P$) is a widely used broad-spectrum herbicide employed for the control of weeds in agricultural fields (Dayan, 2020). It is commercially marketed under trade names such as Roundup. Owing to its extensive application, glyphosate frequently enters aquatic ecosystems through surface runoff and leaching, posing potential risks to non-target organisms including fish. Glyphosate acts by binding to phosphoenolpyruvate, the substrate of the enzyme EPSP synthase, thereby inhibiting the synthesis of aromatic amino acids via the shikimate pathway (Schonbrunn *et al*., 2001; Clair *et al*., 2012). It is an organophosphorus-based, post-emergence herbicide effective against a broad spectrum of plant species. In aquatic systems, glyphosate readily ionizes into anionic forms and can adsorb onto sediments at pH levels above 3.5 (Rzymski, 2014).

Clarias batrachus (Linn. 1758), locally known as "Mangur," is a nutritionally valuable and easily digestible freshwater fish. It is often recommended in the diet of pregnant women, anemic patients, and children (Debnath, 2011). As an air-breathing fish, it is commonly cultured in stagnant waters and forms an integral component of paddy-field aquaculture systems in many regions (Arjun, 2009).

The present investigation was undertaken to evaluate the chronic toxic effects of glyphosate exposure on the biochemical profile of the freshwater fish *Clarias batrachus*.

MATERIALS AND METHODS :

Healthy specimens of *Clarias batrachus* (10–12 cm; 30–34 g) were collected from the local fish market in Darbhanga and acclimatized to laboratory conditions for 15 days after treatment with 0.1% $KMnO_4$ solution. Fish were fed a commercial diet (28% crude protein) at 3% of body weight daily following APHA (1985) guidelines.

The LC_{50} of glyphosate was determined using static acute bioassays, and mortality was recorded at 24, 48, 72, and 96 hours. The 96-hour LC_{50} value was 24.6 mg/L. A sublethal concentration (1/10th of 96-hour LC_{50}), i.e., 2.46 mg/L, was selected for chronic exposure. Twenty fish were exposed to 2.46 mg/L glyphosate for 30 days, while another twenty fish served as control. After the exposure period, liver, kidney, muscle, and gill tissues were dissected and analysed for glycogen (Carroll *et al*., 1956), total protein (Varley *et al*., 1980), and total lipid (Folch *et al*., 1957) using standard biochemical methods.

RESULTS AND DISCUSSION:

TABLE:-1

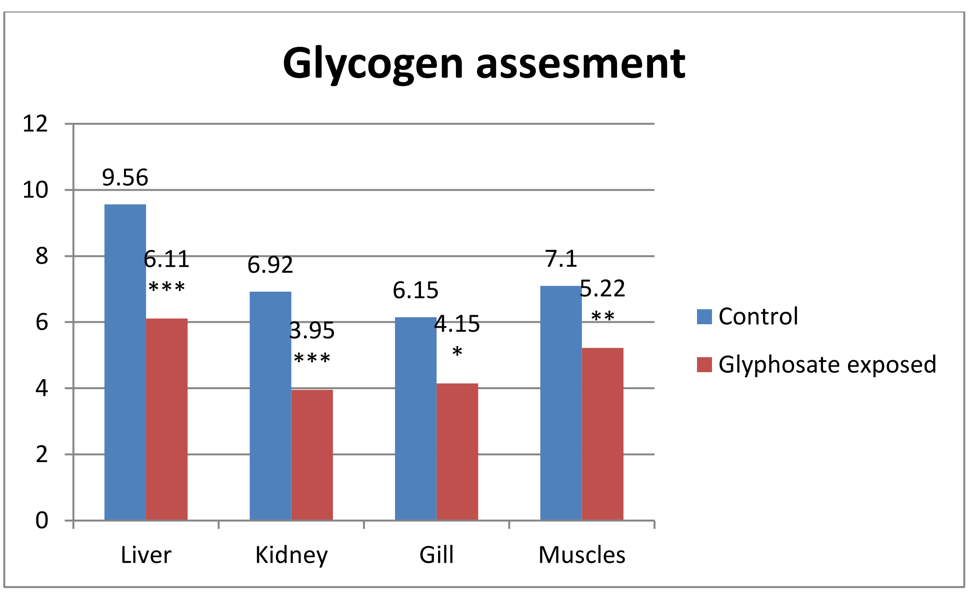
In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes of profile of Glycogen (mg/g wet tissue). Values are mean ± SE of 5 observations.

Tissue	Control	Glyphosate exposed
Liver	9.56±0.53	6.11± 0.53(-36.08%) ***
Kidney	6.92 ± 0.25	3.95 ± 0.25 (- 42.91%) ***
Gill	6.15±0.52	4.15±0.40 (-32.52%) *
Muscles	7.10±0.52	5.22±0.43 (-26.47%) **

*Values indicate percent increase (+), Or decrease (-) over control values significant at , * P<0.05, ** p<0.01*

FIGURE-1

In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes of profile of Glycogen (mg/g wet tissue). Values are mean ± SE of 5 observations.



PARAMETERS

TABLE:-2

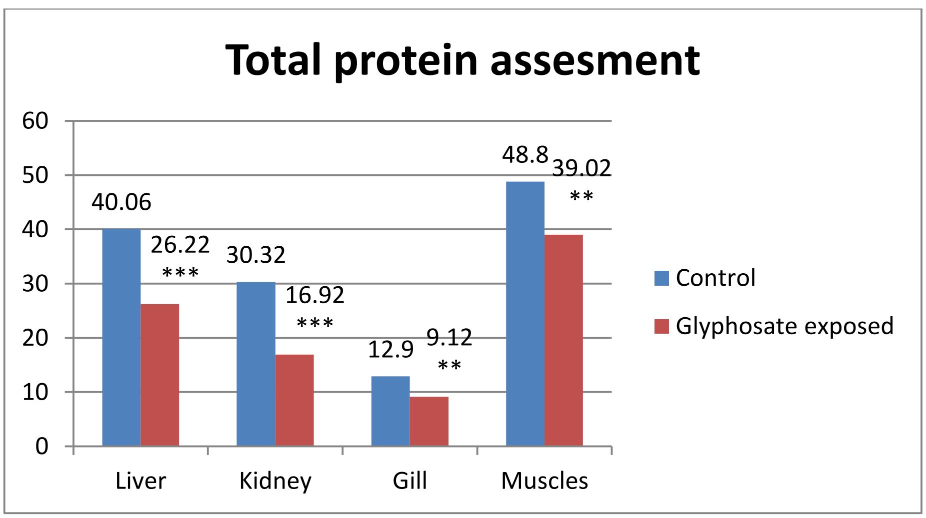
In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes in profile of total proteins (mg/g wet tissue) Values are mean ± SE of 5 observations.

Tissue	Control	Glyphosate exposed
Liver	40.06±1.83	26.22± 0.53(-34.54%) ***
Kidney	30.32 ± 0.22	16.92 ± 0.25 (-44.19%) ***
Gill	12.90±0.52	9.12±1.3 (-29.30%) **
Muscles	48.80±0.52	39.02±1.3 (-20.04%) **

Values in indicate percent increase (+), Or decrease (-) over control values significant at, ** *p<0.01,* *** *p<0.001*

FIGURE:-2

In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes in profile of total proteins (mg/g wet tissue) Values are mean ± SE of 5 observations.



PARAMETERS

TABLE :-3

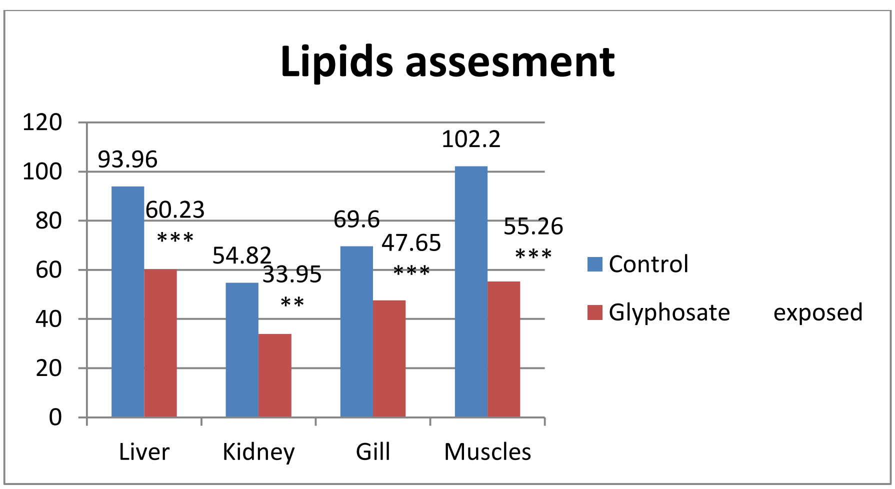
In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes in levels of total lipids (mg/g wet tissue). Values are mean ± SE of 5 observations.

Tissue	Control	Glyphosate exposed
Liver	93.96±1.63	60.23± 0.55 (-35.89%) ***
Kidney	54.82 ± 0.50	33.95 ± 0.2 (-38.07%) **
Gill	69.60±1.02	47.65±0.67 (-31.55%) ***
Muscles	102.20±2.02	55.26±1.67 (-45.92%) ***

*Values in indicate percent increase (+), Or decrease (-) over control values significant at, * p<0.05*

FIGURE-3

In tissue of *Clarias batrachus* exposed to Glyphosate (2.46 mg/l for 20 days) changes in levels of total lipids (mg/g wet tissue). Values are mean ± SE of 5 observations.



PARAMETERS

1. GLYCOGEN IN GILL, LIVER, KIDNEY AND MUSCLES:

The results was explained in table-1. reflect the finding of the present study on glycogen in the Glyphosate exposed fish *C. batrachus*. The glycogen profiles of control groups have been estimated as follow: liver: 9.56±0.83, kidney: 6.92 ± 0.22, gill: 6.15 ± 0.22, and muscles: 7.10±0.52. In the treated fish group a considerable decrease in quantity of glycogen in these organs has been recorded. The glycogen distribution in treated group was found to be as liver: 6.11± 0.53, kidney: 3.25± 0.53, gill: 4.15 ± 0.2 and muscles: 5.22±0.43. Percentage decrease is distributed in order as highest in kidney: (-42.91%) >liver: (-36.08%) > gill: (-32.52%) > muscles: (-26.47%). The value of liver and kidney are showed very significant (at $p<0.001$) and muscles showed significant (at $p<0.01$) but gill has value non significant (at $p<0.05$) (Table-1, Fig.-1). Carbohydrates constitute an important biochemical component of animal tissues and serve as a primary energy reserve, mobilized to meet immediate metabolic demands. Alterations in glycogen content represent a significant biochemical lesion induced by exposure to toxicants. Depletion of glycogen reflects its rapid utilization through glycolysis and the hexose monophosphate pathway to compensate for increased energy requirements in stressed fish. A significant reduction in muscle glycogen levels under toxic stress has also been reported by Sastry and Subhadra (1982). The observed decrease in glycogen levels in various tissues of fish exposed to toxicants is consistent with earlier findings (Ramalingam *et al.*, 2000; Shobha *et al.*, 2007; Bedii and Kenan, 2005; Pawar *et al*., 2009). This reduction may be attributed to stress-induced endocrine responses, particularly the release of adrenaline and noradrenaline, which stimulate catecholamine secretion and enhance the conversion of liver glycogen into blood glucose to meet elevated energy demands (Sriwastava, 1985). Furthermore, toxicant exposure may disrupt oxidative phosphorylation, interfering with the fundamental energy conservation mechanisms of the cell. Impaired oxygen transport can lead to tissue hypoxia and acidosis, thereby promoting glycogenolysis. Under anoxic conditions, glycogen consumption increases to compensate for energy deficits, resulting in a marked decline in tissue glycogen levels (Karat *et al*., 2009; Maurya *et al*., 2016; Mohan, 2017).

2. TOTAL PROTEIN IN GILL, LIVER, KIDNEY AND MUSCLES:

The results was explained in table-2 reflect the findings of the present study on total protein content of different organs in the Glyphosate treated fish, *C. batrachus*. The total protein profiles of control groups have been estimated as follow: liver: 40.06±0.83, kidney: 30.32 ± 0.22, gill: 12.90 ± 0.22, and muscles: 48.80±0.52. In the treated fish group a considerable decrease in quantity of total protein in these organs has been recorded. The total protein distribution in treated group was found to be as liver: 26.22± 0.53, kidney: 16.92± 0.53, gill: 9.12 ± 0.2 and muscles: 39.02±0.43. Percentage decrease is distributed in order as highest in kidney: (-44.19%) >liver: (-34.54%) > gill: (-29.30%) > muscles: (-20.04%). The value of liver and kidney are showed very significant (at $p<0.001$) while muscles and gill have value significant (at $p<0.01$) (Table-2, Fig.- 2). Proteins are primary biomolecules essential for growth, differentiation, and the maintenance of cellular homeostasis. Being integral to both the structural and functional aspects of cells, proteins play a key role in cellular metabolism. The significant depletion of tissue proteins observed in the present study indicates a high catabolic activity of affected organs, likely due to enhanced proteolysis and the utilization of amino acid degradation products for energy metabolism under toxic stress. The reduction in protein content in fish tissues may result from several metabolic processes: the utilization of keto acids in gluconeogenesis for glucose synthesis, the mobilization of free amino acids for protein synthesis, or their involvement in osmoregulation and ionic balance. Protein depletion may also act as a compensatory mechanism under stress, providing intermediates for the Krebs cycle to meet increased energy demands (Schmidt, 1975). Similar decreases in protein levels in fish exposed to various toxicants have been reported in earlier studies (Ramalingam *et al*., 1982; Maurya *et al*., 2016; Mohan, 2017).

3. TOTAL LIPIDS IN GILL, LIVER, KIDNEY AND MUSCELES:

The results was explained in table-3 reflect the findings of the present study on total lipids content in the glyphosate exposed fish *C. batrachus*. The lipids profiles of control groups have been estimated as follow: liver: 93.96±1.83, kidney: 54.82 ± 0.52, gill: 69.60 ± 0.22, and muscles: 102.20±1.52. In the treated fish group a considerable decrease in quantity of lipids in these organs has been recorded. The lipids distribution in treated group was found to be as liver: 60.23± 0.53, kidney: 33.95± 0.53, gill: 47.65 ± 0.2 and muscles: 55.26±0.43. Percentage decrease is distributed in order as highest in muscles: (-45.92%) >kidney: (-38.07%) > liver: (-35.89%) > gill: (-31.53%). The value of liver, muscles and gill are showed very significant (at $p<0.001$) and value of kidney showed significant (at $p<0.01$) (Table-3, Fig.-3). The decrease in lipid levels in fish exposed to various toxicants has been reported in earlier studies, such as in the European eel (*Anguilla anguilla*) exposed to the heavy metal cadmium (Fabien *et al*., 2007). Lipids, including phospholipids, free fatty acids, free cholesterol, diglycerides, and triglycerides, serve as important energy reserves similar to glycogen. In the present study, the lipid content in the liver and muscle tissues of *Clarias batrachus* exposed to sublethal concentrations of glyphosate was significantly reduced, consistent with observations in fish exposed to other toxicants (Mishra and Srivastava, 1983; Levesque *et al*., 2002; Fabien *et al*., 2007; Sobha *et al*., 2007; Dilip and Vidya, 2016; Mohan, 2017).

Lipid depletion was more pronounced in the liver than in the muscles, likely due to enhanced lipolysis of structural and storage lipids to maintain essential metabolic processes under toxic stress, thereby disrupting normal homeostasis. Additionally, a significant increase in serum glucose levels may result from enhanced glycogen breakdown via glycogenolysis. Elevated levels of free cholesterol in the serum were also correlated with decreased liver lipid content, reflecting mobilization of lipid reserves under glyphosate-induced stress (Pratibha, 2013; Maurya *et al*., 2016).

CONCLUSION:

Clarias batrachus with average weight 30.0± 4.0 g has susceptible to herbicides, glyphosate at low concentration as 2.46 mg/l. The chronic toxicity can be detected through biochemical alteration. So, suggested it may the less than above concentration be suitable for fish culture, optimum growth performance and survival rate than other water conditions. The biochemical parameters may be used as a tool to detect the herbicides toxicity to fish culture monitoring.

ACKNOWLEDGEMENT: *The authors are thankful to the Department of Zoology, C.M.Sc. college, LNM University, Darbhanga, Bihar for the provision of laboratory facilities used in this study.*

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