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Effects of herbicide, glyphosate on behaviour response & blood metabolite of *Clarias batrachus* (Linn.)

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Abstract
The present investigation includes the blood biochemical alterations in chronic (30 days) exposure of the airbreathing fish, *Clarias batrachus* (Linn.) induced by sublethal concentration (2.46 mg/L) of inorganic herbicide, glyphosate. Significant changes were observed in the blood biochemical properties in the form of hyperglycemia, hypoproteinemia and hypercholesterolemia. The present study therefore points towards a severe metabolic dysfunction in response to herbicide, glyphosate toxicity in the fish Clarias batrachus. So, it is suggested that more suitable to culture at water glyphosate concentration of < 2.46 mg/L for optimum growth performance and survival rate than other water conditions.

Keywords: Herbicide, glyphosate, metabolite, *Clarias batrachus*, hyperglycemia, hyperproteinemia, hypercholesterolemia

Introduction
Pesticides are used worldwide in a variety of applications. Pesticide contamination of aquatic system has attracted the attention of researchers and has increased in the last decades due to extensive use of them in agricultural, chemical and industrial processes which are becoming threats to living organisms (Jerald & Saradhamani, 2015) [11]. It is reported that approximately three million people are poisoned and 200,000 die each year around the world from pesticide poisoning; the majority of them from developing countries (FAO, 2000) [9]. Glyphosate is one of the extensively used worldwide agricultural herbicide. It is a post emergence herbicide, which acts by binding phosphoenolpyruvate, the substrate of EPSP synthase, and subsequently inhibiting aromatic amino acid synthesis via the shikimate pathway in plants. Glyphosate is commonly applied as part of a formulated product, the most widely used of which are the Roundup herbicides. Glyphosate present in the form of an isoproylamine salt in Roundup formulation, which aids solubility without affecting its properties as the active ingredient, together with various adjuvants which enhance its herbicidal properties. The commonly commercial used adjuvants is polyethoxylated tallow amine (POEA), a surfactant that enhances penetration of glyphosate through the plant cuticle. However, contamination can be expected when rainfall occurs directly after application and when river flooded (Le Mer, *et al.*, 2013) [18]. The use of herbicides in agricultural practices is on the increase, thereby affecting ecosystems and leading to ecological imbalance. Fishes are vital indicators of the effects of toxic compounds in aquatic toxicology (Ayanda, *et al.*, 2015) [4].
This study aimed to examine the effects of glyphosate on blood biochemical in fish and to determine to what extends this effect were associated with the toxicity of glyphosate alone. Hence, in the present study efforts have been made to illustrate the blood biochemical alterations induced by exposure herbicide, glyphosate on freshwater fish, *Clarias batrachus.*

Materials and Methods
The snakehead fish, *Clarias batrachus* procured live from the local fish market, Darbhanga were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (15±2.0 cm) and weight (30±2.2 g) were acclimated for 15 days to laboratory conditions. Commercial diet containing 28% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the morning (10.00 AM). Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of glyphosate (41% manufacture from Gujrat, India) the mortality was recorded after 24, 48, 72 and 96 hr, and were calculated by the Finney method (1978) [8]. The LC_{50} values for these periods were 24.6 mg, 27.4 mg, 31.9 mg and 34.7 mg respectively. 1/10th value of the LC_{50} value for 96 hr was taken as the sublethal concentration (Sprague, 1971) [30]. Twenty acclimated fish were exposed to a sub-lethal concentration (2.46 mg) of glyphosate for 30 days. Side by side same number of fish as that of experimental one was maintained as the control group. However, mortalities were removed immediately, and behavioural abnormalities were assessed at these regular intervals following a modified behavioural protocol checklist Klesius *et al*. (2000) [16]. Scores were assigned daily to individual fish in the experiment and were based on the following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or jerk moement, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 30th day blood samples was extracted from the caudal dorsal of the test fish and were then processed for quantitative estimation of blood glucose (Sinha, 1990) [29], serum protein ('Biuret method' of Varley *et. al.*, 1980) [32] and serum cholesterol (Kabara's method, 1966) [14].

Result

Behavioural response

The control fish shows a tendency to remain at the bottom of the aquarium with little disturbance. Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hr exposure of glyphosate. After 48 hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Thin film of mucous with gill adhesion was noticed on gills, operculum and general body surface at this stage. After 72 hr exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. After 96 hr ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost their natural colouration. Loss of equilibrium before death settle down at bottom is a symptom shown all the test fish.

Blood biochemistry studies

There are the three principal constituents of the cell i.e. carbohydrates, proteins and lipids. These are also called metabolites and play a major role as energy precursors for fishes exposed to stress conditions. The optimum concentrations of these biomolecules are required for the proper and normal physiologic function of a cell. Any alteration in their organization or concentration may therefore, disturb the normal physiology of the cell.

Blood metabolite levels

The variables monitored at blood metabolites levels are blood glucose, serum protein and serum cholesterol of experimental fish *Clarias batrachus.* On exposure to a sub-lethal concentration (2.46 mg/l) of glyphosate the fish reveals following changes in the biochemical parameters.

Blood glucose

The blood glucose level in the control fish group is assessed to be 65.66±0.83 mg/100ml of blood. The experimental fish become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood (87.82± 1.30 mg/100ml) which counts to an increase of 28.53% (Table-1) the present values of the present observations are expressed as meant S.E. of 5 fish in each group.

Serum protein

Contrary to the elevated blood glucose level the experimental fish group show highly significant ($P< 0.001$) depletion in the level of serum protein in control group estimated to be 6.05 ± 0.40 g/100 ml as against 3.59 ± 0.10 g/100 ml in the treated group (Table-1).

Serum cholesterol

The serum cholesterol level in the fish of control group has been analysed 202±2.06 mg/100 ml. The treated fish show hypercholestrolemic response as evident by significant ($p< 0.05$) increase (215.8±1.95 mg/100 ml) in its level (Table-1) the serum cholesterol has been found to increase by 13.01% in the present case.

Table 1: Changes in the blood / serum metabolite levels in *Clarias batrachus* exposed to glyphosate (2.46 mg/l) for 30 days. Values are mean ± SE of 5 observations.

Parameters	Control	Glyphosate exposed
Blood glucose (mg/100 ml)	65.66±0.83	87.82± 1.30(+28.53)
Serum protein (g/100ml)	6.05 ± 0.40	3.59 ± 0.10 (- 2.38)
Serum cholesterol (mg/100 ml)	202±2.06	215.8±1.95 (+13.01)

Values indicate percent increase (+) or decrease (-) over control values significant at $*P<0.05$, $***p<0.001$

Discussion

In the present study, certain deformities and unusual swimming patterns, jerk movement were found in fish exposed to 2.46 mg/L of glyphosate and above concentrations. The results of the present study also indicate that the fish exposed with sublethal concentration to this herbicide getting chronically stage. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Paul and Banerjee, 1996 [23]; Palanivelu *et al.*, 2005 [21]; Ufodike and Onusiriuka, 2008) [31]. Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1973) [7]. Acute toxic effect mercuric chloride was observed on *H. fossilis* by Pathak P. & Anand, (2020) [22]. The toxic effects of surfactant, dodecyl dimethyl benzyl chloride (1227) on larval locomotors of zebrafish was observed by Yanan, *et al.* (2015) [34]. It is, therefore, conclude that the toxicity of the herbicide, glyphosate depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for herbicide toxicity to fish culture monitoring.

Blood glucose

Blood glucose level in serum of *Clarias batrachus* after treated with different concentration of glyphosate showed highly significant increases ($P<0.001$) (87.82± 1.30 mg/100ml), in all groups compared to the control fish group (65.66±0.83 mg/100ml). Glyphosate an effect is known to increase the levels of activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. Blood glucose increased due to stimulation of glucocorticoids in stressed catfish. Recently Jha (2009) [12] and Poonam *et al.* (2010) [24], Clair, *et al.*(2012) [5], Koley (2013) [17], Le Mer, *et al.* (2013) [18] and Mohan, (2020) [20] have observed similar result under the exposure of Nuvan, fenvalerate, endosulfan, Atrazine, Eklaux and Glyphosate in various fresh water fishes. Changes in blood glucose concentration is the most widely recognized and consistent response to stressor.

The present study also suggests the involvement of adrenal pituitary glucocortcoied axis and the depletion of energy stores and resultant debility under glyphosate exposure.

Plasma protein

In the present investigation depleted levels of plasma proteins was observed. Serum proteins appear to be very sensitive to glyphosate exposure as is evident by the apparent fall (39.6%) in its content. The decrease in serum protein as observed during the present study suggests that the detoxification/ degradation of the toxicant either took place partially in the blood itself or involved the serum protein. This depletion further suggests a progressive protein degradation or biochemical transformation of the protein Nitrogen into other Nitrogenous products as suggested by Jha (2009) [12], Clair, *et al.* (2012) [5], Koley (2013) [17], Le Mer, *et al.* (2013) [18], Ajima *et al.*,(2015) [3], Ahsan (2016) [2], and Pathak, (2020) [22] has observed similar result under the exposure of various toxicants Nuvan, fenvalerate, Atrazine, Eklaux heavy metals in various fresh water fishes.

Significant decrease in total plasma protein was recorded and it was found that the percent decrease was higher in 120 days exposure period (68.2%) then 96 hour (46.4%) in fish *Clarias batrachus* exposed to 11.12 ppm sublethal concentration of cadmium (Sastry & Shukla 1990) [28]. In present study agreement with Clair, *et al.* (2012) [5] decrease in plasma protein within 30 days of exposure of glyphosate at sublethal concentration to *C. batrachus* was recorded.

Serum cholesterol

The present investigation exhibited hypercholesterolemia (Table-1) under the toxic influence of glyphosate and in agreement with the findings of Verma *et al.*(2002) [33]; Prakash & Maheshwari, (2005) [25]; Kalaivani *et al.*,(2008) [15] and Jha & Jha, (2010) [13]; Abedi *et al.*, (2013) [1]; Le Mer, *et al.* (2013) [18], Rani, *et al.* (2015) [27], and Dilip, and Vidya (2016) [6] has observed similar result under the exposure of Nuvan, fenvalerate, Eklaux, Atrazine and heavy metals in various fresh water fishes.

The elevated blood cholesterol level may be due to the hypermetabolic state of the fish or due to the impaired liver function as suggested by Holmberg *et al.* (1972) [10]. Again the increase in cholesterol may be due to its increased synthesis in the liver as the precursors for interval hormones caused by the toxicant. Part of this increased cholesterol store might have found its way into the blood as suggested by Madhavan & Elumalai (2016) [19].

Conclusion

It could be concluded that *Clarias batrachus* with average weight 30.0± 2 g, were more suitable to culture at water herbicide, glyphosate concentration of < 2.46 mg/l for optimum growth performance and survival rate than other water conditions. Significant changes were observed in the blood biochemical properties in the form of hyperglycemia, hypoproteinemia and hypercholesterolemia. Therefore, it can be recommended to be carried out under the similar experimental conditions.

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