HISTOLOGICAL EFFECT OF SUBLETHAL CONCENTRATION OF GLYPHOSATE BASED HERBICIDE IN THE INTESTINE OF *CTENOPHARYNGODON IDELLA*

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Abstract

The present study aims to assess the histological changes in the intestine of fish *Ctenopharyngodon idella* exposed to the herbicide Glyphosate (Roundup). Glyphosate is a broad-spectrum herbicide used to control a wide variety of weeds and plants. The mode of action of glyphosate is to prevent an enzyme pathway, and preventing plants from synthesizing three aromatic amino acids. Healthy fishes irrespective of sex of uniform body weight (30 ± 5 gm.) and body length (12 ± 1.4 cm) were exposed to various concentrations (0.001%, 0.003%, 0.005% and 0.007%) to determine LC_{50} 96 hrs and was calculated as 0.003%. Histopathological study conducted on fish exposed to sub-lethal concentration ($1/2^{th}$) for a period of 10 days. The pathological changes observed in the fishes are ulceration (**UE**) of gastric epithelium and inflammatory cell infiltration (**IN**).

Key words: *Ctenopharyngodon idella*, Glyphosate, Histopathology, Intestine.

Environment pollution is one of the most important problems in our world. It means any solid liquid or gaseous substances present in such concentration as may be, or tent to be detrimental to environment. It also indicate that the presence of any environmental pollutant and the release of potentially harmful substances into the environment due to man's activities. Environmental pollution by toxicants has become one of the most important difficulties in the world. Due to anthropogenic activities large amounts of foreign substances enter in the environment and aquatic environment is the ultimate sink for all pollutants which affect the living organisms (Tilak K.S. and Kumari R. S., 2009). It is also noticed that the fresh water ecosystem occupies an extremely small area and degrade at a very large scale, due to water pollution Ahmed *et al*., (2011). Polluted water not only affects the life of present generation but it also affects the life of upcoming generations because its effect remains for long. A larger part of the contaminants have bio magnification and bioaccumulation capabilities with a broad spectrum of impacts, effects and stresses on aquatic dwelling organisms (Censi *et al.*, 2006).

Aquatic organisms accumulate pollutants directly from contaminated water and indirectly through the food chain (Nussey *et al*., 2000; Ashraf, 2005; Riba *et al*., 2004). The toxicant enters the body of the fish they may affect the organs leading to physiological and pathological disorders. Hence the haematological and histopathological studies are the effective and sensitive tool to analyse the effect of toxicants on various target organs of fish in laboratory experiments and in field investigations (Kori-Siakpere *et al*., 2005). According to Rashed, 2001 fishes are considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level. Several

commercial and edible species have been widely investigated to detect the presence of hazardous chemicals (Begüm *et al.*, 2005).

According to Abdallah and Abdallah, 2008 histopathological fluctuations are observed among fishes exposed to pollutants both in field and in laboratory conditions. Histological studies have been considered as the tool for evaluating the toxic effects in target organs of fish in laboratory experiments and in the field experiments (Dutta, 1996). Greig *et al.,* 2005 reported that the quality of water and the well-being of fishes are interconnected and directly proportional. The fluctuations in any of the parameters severely affect abode organisms, especially fishes. Fish live in close contact with their environment, and are more susceptible to physical and chemical changes in the environment which may be reflected in their blood constituents. Therefore slight differences in water quality subjected to a wide variety of stresses among fishes because their homeostatic mechanisms are highly reliant on existing conditions in their immediate surroundings parameters (Nussey *et al*., 1995). Thee toxicants are generally taken up through different organs of the fish because of the affinity between them and they are concentrated at different levels in different organs of the fish body (Rao & Padmaja, 2000).

Glyphosate has used as the leading herbicide for the control of annual, perennial weeds and volunteer crops in a wide range of different situations and was initially registered as a broad-spectrum, non-selective, systemic herbicide for certain non-crop and plantation crop uses. At present it is most commonly used herbicide with the introduction of genetically modified (GM) glyphosate-resistant (GR) crops (Woodburn, 2000). The histopathological change due to stressors is a useful tool to assess the impact of the toxicity of xenobiotic in vital functions of a living organism.

MATERIALS AND METHODS

Roundup (RD)

Glyphosate under the trade name Roundup® was introduced in the marked by Monsanto Company during the 1970s. Glyphosate is the active ingredient in the herbicide products Roundup (41%). It is a broad-spectrum herbicide used to control a wide variety of weeds and plants. It also used for the control of emerged and shoreline weeds, such as cattail, reed, rushes, smartweeds and some floating-leaf plants like water lily, lotus. Glyphosate, N-(phosphonomethyl) glycine is a systemic and non-selective herbicide used to kill broad leaved, grass, and sedge species. It interferes with cellular processes important for normal plant functioning. Only plants and microorganisms have these specific cellular processes. The mode of action of glyphosate is to prevent an enzyme pathway, the shikimic acid pathway, preventing plants from synthesizing three aromatic amino acids. It is usually applied to the plant and not directly to the water. It is quickly bound to suspended particles and bottom sediments and is rapidly activated. Laboratory assay study showed that Mice, fed moderate doses for 2 years, developed an increase in the number of kidney tumours. Roundup also affects enzymes found in mammals. Symptom of exposure to Glyphosate include eye irritation, burning eyes, blurred vision, skin rashes, burning or itchy skin, nausea, sore throat, asthma and difficulty breathing, headache, lethargy, nose bleeds and dizziness.

Experimental Animals

The fish selected for the present study was ***Ctenopharyngodon idell*a** belonging to the family Cyprinidae (Fig. 1). The grass carp is a relatively large fish that possesses a similar body structure common to other carp species. It can grow up to 1.6 meters in length and weigh up to 37kg making it one of the largest members of the Cyprinidae family (Conover *et al*., 2007). The fish for the present study was collected from freshwater ponds of Alapuzha District. The fishes were kept in glass aquaria 250 L capacity for a period of four weeks. During the period of acclimatization the fishes were fed with standard food pellets and were exposed to the natural day and night cycle. Healthy fishes with

active movement were considered for the experimentation. The acclimatization was done at 28.83±2ºC room temperature.

Fig. 1 ***Ctenopharyngodon idell*a**

Experimental Protocol

The test fish were starved for 24-h prior to and during the 96 hrs test period and laboratory assay was conducted to determine 96 hrs. LC_{50} values for ***Ctenopharyngodon idell*a** exposed to the herbicide. The test was carried out in 90 x 60 x 30 cm rectangular tank with ten healthy fishes in triplicate, controls were also maintained. The test medium changed every day in order to remove the metabolic waste (Ammonia). The experimental design and calculations for the toxicity analysis were based on the procedure given by Finney (1978) and the computerized programme (SPSS Ver.19). Healthy fishes irrespective of sex of uniform body weight (30 ± 5 gm.) and body length (12 ± 1.4 cm) were selected for the experimental study. The fishes were divided into Test and control group, with ten fishes in each group and maintained without feed for 12 hours before the exposure to effluent. The fishes in test groups were experimentally exposed to concentrations 0.001%, 0.003%, 0.005% and 0.007% respectively, under controlled conditions in aquarium water. Control fishes were maintained in tap water without herbicide. Three sets of experiments for each group were also conducted and the test was performed by the semistatic (renewal) method in which the exposure medium was exchanged every 24 h to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment. From the test the LC_{50} calculated. Histopathological study conducted on fish exposed to sublethal concentration ($1/2^{th}$) for a period of 10 days. From the experimental and control group fishes, the intestine of the fishes carefully removed and processed in 10% natural formalin. The tissue samples were processed and the histological changes were noted.

Histopathological analysis

For histopathological examinations intestine is isolated from control and experimental fishes through trans spinal dissection, gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and adhering debris and immerse in fixative sera composed of glacial acetic acid , formaldehyde and ethanol (1:3:7). The fixed tissues were passed through the alcohol series 50 to 100% for dehydration and finally cleared in chloroform. Tissues were then impregnated with paraffin wax (58ºC) and were embedded. After embedding the blocks were subjected to sectioning at 5µ in thickness in a rotary microtome (Leica Rotary type). The sections were stained with Haematoxylin and Eosin stains. The stained slides were treated with Xylene and mounted in DPX. The stained sections were observed under a research microscope attached with digital camera (Olympus E420 camera) and photographed.

RESULTS AND DISCUSSION

LC_{50} - 96 hrs

The lethal concentration of roundup for 50% fish death (LC_{50} - 96 hrs exp.) was calculated as 0.003%.

Histopathological changes in Intestine

Normal intestine with three distinct layers they are mucosa layer (MU) lined by gastric epithelium (GC), sub mucosa (SM) and muscle coats (MC) (Fig. 2). The pathological changes were observed in the GI tract include ulceration (UE) of gastric epithelium and proliferation. All the three layers were infiltrated with acute inflammatory cells (IN). Damage of columnar epithelial cells. Shrinkage of columnar epithelial, disintegration of the cell wall also noticed in the experimental compare to control fish. A similar observation noticed in intestine of the fish *Oreochromis mossambicus* exposed to 192 hrs. Sub-lethal exposure of cypermethrin at 0.008 ppm (Thayappan Karthigayani1 *et al*., 2014). Herbicide treated fish intestine revealed severe atrophic degeneration. The mucosa lost its usual shape. Since villus is the working unit of intestine, in the present study they show altered orientation, greatly reduced and their tips were found ruptured.

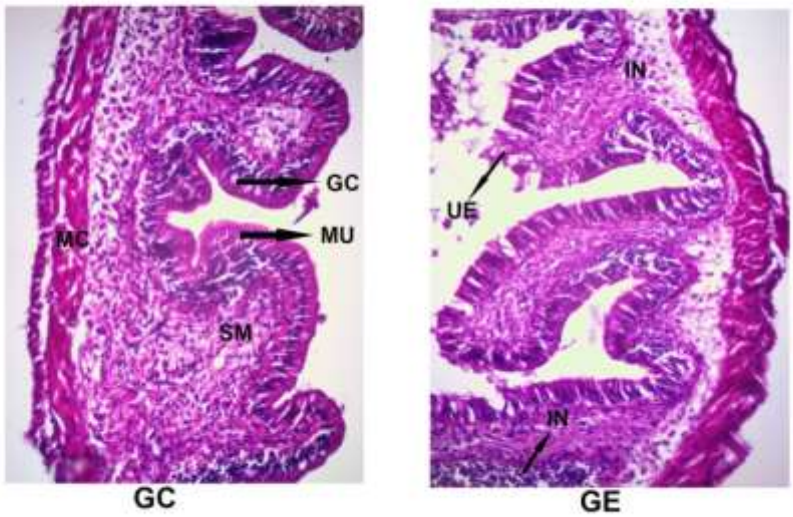

Fig. 2 Histology of Intestine

MU	:	Mucosa layer	**GC**	:	Gastric epithelium
MC	:	Muscle coats	**UE**	:	Ulceration
SM	:	Sub mucosa	**IN**	:	Inflammatory cell infiltration

SUMM ARY AND CONCLUSION

Glyphosate (Roundup) is commonly used herbicides that could be persistent and mobile in soil and water, and it is known to be one of the most common terrestrial and aquatic contaminants (Cox, 1998). This study revealed that glyphosate herbicide is toxic to *Ctenopharyngodon idell*a and causes histopathological changes in Intestine. The pathological changes were observed in the GI tract include

ulceration of gastric epithelium and proliferation, acute inflammatory cells. Therefore the present investigation indicated that the use of glyphosate herbicide near the aquatic environment should be discouraged. The study also suggested the urgent need to encourage natural substances to eliminate the unwanted weeds for protecting fish and man.

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