Effect of acute exposure of herbicide pendimethalin on tissue protein levels in freshwater fish *Channa punctata* (Bloch)

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ABSTRACT: Pendimethalin is a widely used herbicide in agricultural field to control weeds in many areas of India. Chemicals like herbicides; pesticides etc. after releasing from various agricultural fields impair water quality directly or indirectly causing severe damage to the aquatic ecosystem especially to fishes. In the present study, the toxic effect of pendimethalin was evaluated by measuring tissue protein levels in a commercially important fish, Channa punctata. Adult healthy fishes were used and divided into 5 experimental groups; first group was kept as control, second one as DMSO (solvent) exposed, while fishes of third, fourth and fifth groups were exposed to 10% (0.220 mg/L), 20% (0.440 mg/L) and 30% (0.660 mg/L) of 96 h LC_{50} value of pendimethalin respectively, for duration of 96 hours in a semi static system. The results revealed a significant decrease in liver, gonad and blood protein levels in the exposed fishes in a dose and time dependant manner when compared to control. The occurrence of decreased protein level in the observed tissues of the treated fishes is the indications of dysfunctional protein metabolism in response to the test chemical.

Keywords: Pendimethalin, Channa punctata, acute, protein, liver, gonad.

1. Introduction:

The contamination of water bodies with continuous release of pollutants including different pesticides, herbicides, industrial wastes etc. from various agricultural fields and industries has become a matter of great concern for the health of aquatic organisms (Wagenhoff *et al.*, 2011), especially for the fishes as they are very sensitive to wide range of toxicants (Heger *et al.*, 1995). Application of herbicides in weed management has been recognized as a common agricultural practice throughout the world. Unfortunately, due to the uncontrolled spraying of herbicides to improve agricultural production and yields, aquatic environments are highly polluted triggering a great impact on non target organisms and their environments (Malins and Ostrander, 1991).

Channa punctata is one of the most common freshwater fishes used in toxicological studies because it shows a number of ecotoxicological characteristics such as wide distribution in freshwater environment, availability throughout the year, easy acclimatization to laboratory conditions and commercial importance. These make this species an appropriate model that can be used as indicator species for toxicity and biochemical studies (Pandey *et al.*, 2005).

Herbicide pendimethalin [N-(1-ethylpropyl)- 2,6-dinitro-3,4-xylidine] is a broad spectrum chemical belongs to dinitroaniline family, used primarily in agricultural fields for the control of annual grasses and certain broadleaf weeds in commercial crops (Engebretson *et al.*, 2001, EI-Sharkawy *et al.*, 2011). It is a moderately to extremely toxic systemic toxicant that induces toxicity to fishes and other aquatic organisms which can reportedly facilitate the formation of carcinogenic nitrosamines, a group C carcinogen (possible human carcinogen) (US EPA, 1997).

Presence of herbicides in the vital tissues of target species induces biochemical and physiological alterations causing disturbances in the normal metabolism as well as inhibition of some important enzymes. Many researchers have been worked out regarding the alterations of biochemical contents in different tissues of fishes in response to various agrochemicals and heavy metals (Remia *et al.*, 2008; Hadi *et al.*, 2009; Ganeshwade 2011). Apart from biochemical changes, it has been reported that bioaccumulation of such toxic pollutants adversely affect various tissues of fishes by inducing physiological disturbances, haematological alterations, reproductive abnormalities, hormone disruption, immune suppression, behavioural alteration on *Raibow trout, Chinook* and *salmon* (Mitchell *et al.*, 1987), *Salmon, daphnia* and *trout* (Servizi *et al.*, 1987), *Cyprinus carpio* (Neskovic *et al.*, 1996), and Nile tilapia (Jiraungkoorskul *et al.*, 2002). Determination of toxicity is essential for evaluating the degree of damage to the target organs that produce adverse effects not only to the exposed organisms but also to the other organisms including human beings (IARC, 1993). Thus, it is essential to obtain information regarding the toxicity of such chemicals so as to formulate the proper strategy for safe guarding aquatic organisms.

Data on pendimethalin and its toxicity on fish and other aquatic organisms are very limited. The present work was designed to investigate the impact of acute sublethal doses of pendimethalin (EC 98.8%) on protein contents of liver, gonad and blood of *Channa punctata* under laboratory conditions, which may be useful for understanding the mechanism of pendimethalin toxicity and its management.

2. Materials and methods:

2.1. Test chemical and reagents:

The herbicide pendimethalin (98.8% EC 254-938-2) with CAS R. No. 40487-42-1 was selected as a test chemical for the present study purchased from Sigma Aldrich Corporation (USA). Stock solution of the test chemical was prepared by dissolving 10 mg of pendimethalin in 1 mL of DMSO. Three different test concentrations were prepared by diluting the solution with appropriate dechlorinated tap water for the protein estimation bioassay. DMSO and all other chemicals were purchased from scientific distributors of Guwahati, Assam. All reagents were of analytical grade.

2.2. Test animal and its maintenance:

Adult healthy *Channa punctata* of both sexes with an average body size of 18±3.2 cm and body weight of 80±5.0 g were obtained from local sources and were treated with 0.5% $KMnO_4$ solution for two minutes to avoid any dermal infection (Pandey et al., 2009). The fishes were acclimatized in a laboratory environment for 20 days prior to the experiment to a semi static system in glass aquaria (80×40×30

cm capacity) filled with 60 litres of de-chlorinated tap water under normal day and night condition. During acclimatization fish were fed daily with commercial dry food pellets (Tokyo pellets). Before and during the experiment physiochemical properties viz. temperature (24.40-25.50 ºC), DO (7.20-7.80 mg/L), P^H (7.40-7.90), FCO_2 (0.72-0.81 mg/L) and total alkalinity (90.45-98.60 mg/L) of aquaria water were determined according to APHA / AWWA / WEF (1998).

2.3. Experimental design:

Fishes were divided into 5 equal groups with two replicates (10 fish replicate^{-1}). The first group was maintained in tap water as control, second group as DMSO exposed, while third, fourth and fifth groups were exposed to three sub-lethal concentrations of pendimethalin which were 10% of LC_{50}= 0.220 mg/L, 20% of LC_{50}= 0.440 mg/L and 30% of LC_{50}= 0.660 mg/L; respectively, for 96 hours. Feeding was stopped 24 hours prior to the experiment as recommended by Ward, Parrish (1982) and Reish, Oshida (1987). No mortalities observed during the exposure period.

2.4. Tissue sampling and biochemical analysis:

Fishes of each group were anesthetized with MS222 (Ethyl 3-amino benzonate methanesulfonate) at the end of every 24 hours, 48 hours, 72 hours and 96 hours and blood samples were also collected from caudal vein (Luky, 1977) using sterile non-heparinised syringes into Eppendorf tubes and centrifuged at 3000 rpm for 10 minutes at 4ºC for serum separation and preserved at -20ºC till to be analyze. Tissues (liver, testis and ovary) were also dissected out carefully, rinsed in ice-cold 0.8% NaCl, blotted and weighed, homogenized for 5 minutes using an electric tissue homogenizer and were centrifuged at 10000 rpm for 20 minutes at 4ºC. Protein levels were estimated by Folin-phenol reaction method as described by Lowry *et al.*, (1951) using BSA (Bovine serum albumin) as standard. Values have been expressed as mg/g of liver and gonad tissues and g/dl of blood.

2.5. Statistical analysis:

The data obtained were expressed as Mean ± SEM. The means were subjected to Student's t-test to determine the significant differences at 95% and 99% ($P<0.05$ & 0.01) confidence level using SPSS computer statistical software (version 21).

3. Results:

The result showed significant alterations in protein levels on the targeted tissues of *Channa punctata* exposed to sub-lethal doses of pendimethalin when compared to the control (Figure 1-4). Liver protein level was significantly decreased at all the concentrations of pendimethalin in the experimental groups (Fig. 1). At highest concentration (0.660 mg/L) of pendimethalin exposure protein content was observed to decrease with percentage of 5.35%, 9.18%, 17.89% and 40.17% at 24 hours, 48 hours, 72 hours and 96 hours respectively as compared to control.

The changes in the testicular protein levels are shown in Fig. 2. Protein content was observed to increase during the first 24 hours of exposure at low and medium doses (0.0220 mg/L and 0.440 mg/L) over control which is found to be non-significant. But after 72 hours and 96 hours of exposure, concentration and time dependant decrease in protein level was observed at all concentrations. The percentage decrease in protein content at 96 hours were 9.93%, 17.65% and 27.57% in the fish exposed to 0.220, 0.440 and 0.660 mg/L concentration respectively over the control.

The activities of protein in ovary follow the same pattern as in the liver of pendimethalin exposed fishes. Significant decreases in ovarian protein level in the exposed groups were observed (Fig. 3) when compared to the control. Maximum decrease in ovarian protein level was observed at 96 hours when fishes were exposed to heavy dose (0.660 mg/L) of herbicide solution,

Alterations in total blood protein levels at different hours of chemical exposure are shown in Fig. 4. The percentage decrease of blood protein level at 96 hours was highest (43.48%) in the fishes treated with 0.660 mg/L of test chemical and least (13.83%) in the group exposed to 0.220 mg/L. No significant difference has been observed between control and DMSO (solvent) exposed groups throughout the experiment.

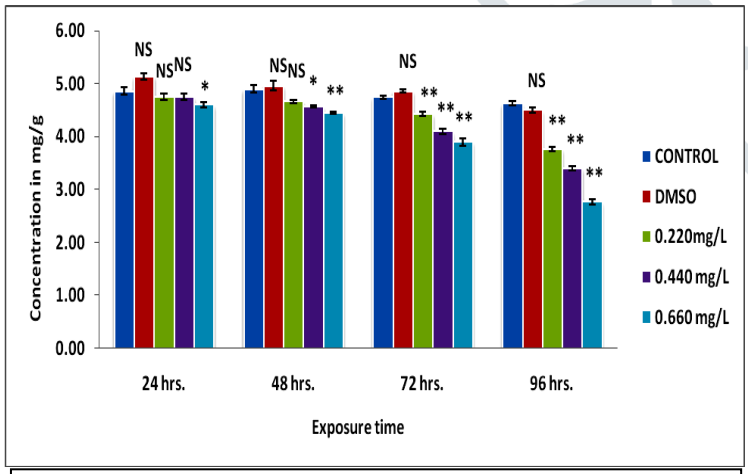

Figure 1: Protein content (mg/g) in liver of *Channa punctata* exposed to 0.220, 0.440 and 0.660 mg/L (10%, 20% and 30% of 96 h LC_{50} value) of Pendimehalin for different time exposures.

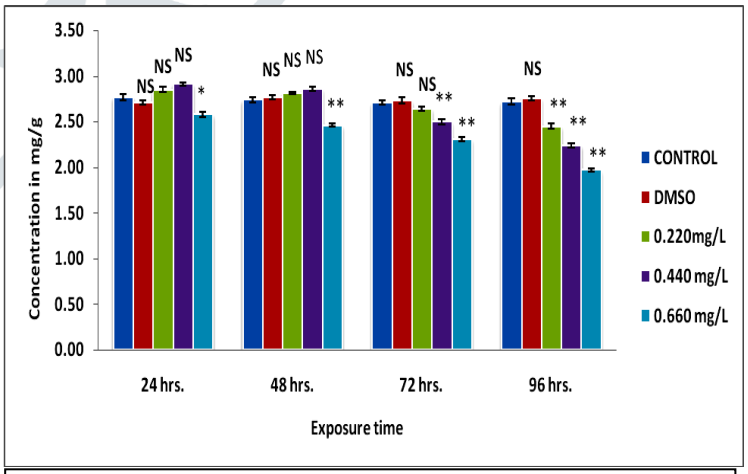

Figure 2: Protein content (mg/g) in testis of *Channa punctata* exposed to 0.220, 0.440 and 0.660 mg/L (10%, 20% and 30% of 96 h LC_{50} value) of Pendimehalin for different time exposures.

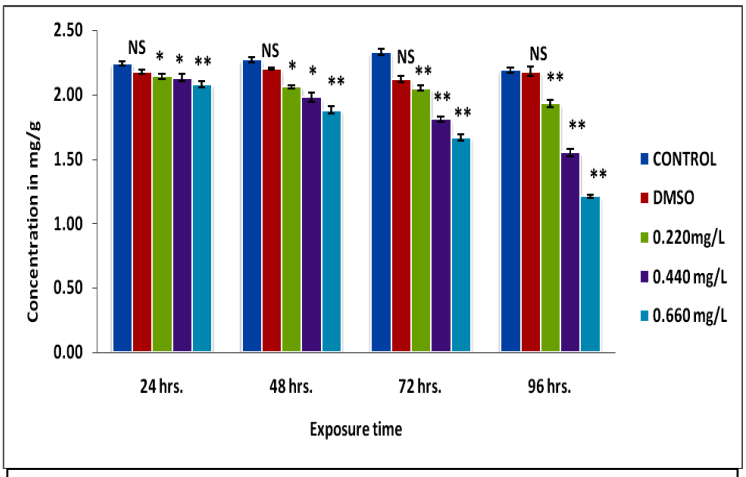

Figure 3: Protein content (mg/g) in ovary of *Channa punctata* exposed to 0.220, 0.440 and 0.660 mg/L (10%, 20% and 30% of 96 h LC_{50} value) of Pendimehalin for different time exposures.

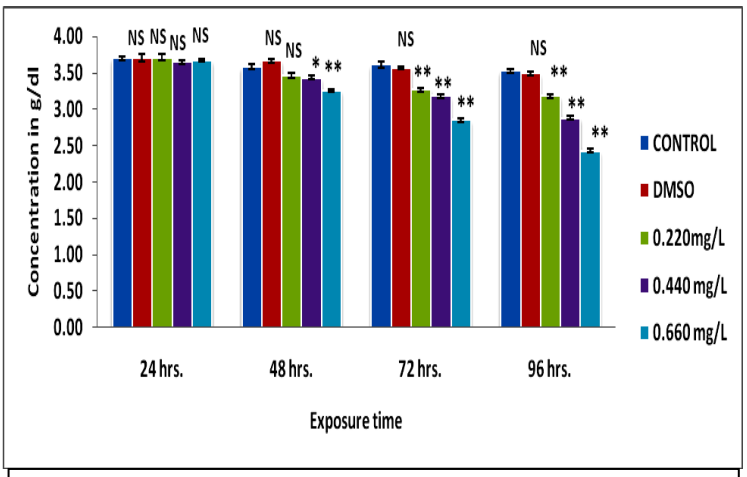

Figure 4: Protein content (g/dl) in blood of *Channa punctata* exposed to 0.220, 0.440 and 0.660 mg/L (10%, 20% and 30% of 96 h LC_{50} value) of Pendimehalin for different time exposures.

(All data are presented as Mean ± SEM, n= 10. * = Significantly different from the control with P< 0.05, ** = significantly different from control with P< 0.01, NS= non significant).

4. Discussion:

The current study showed significant reduction in protein levels on all the targeted tissues of *Channa punctata* exposed to sub-lethal doses of pendimethalin which contributes to the assessment of toxicity of the experimental herbicide on this fish species. The probable cause of decreased protein content observed in the present study may include the metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose or the maintenance of osmotic and ionic regulation (Schmidt Nielson, 1975). During stressful condition animals require high energy to detoxify toxicants and to overcome stress which might have led to the stimulation of protein catabolism initiated by increased proteolysis (Schmidt, 1975; Murthy *et al.*, 1982; Muley *et al.*, 2007; Mamata Kumari, 2007; Chezhian *et al.*, 2010;). Inhibition of metabolizing enzymes by toxicants exposure might also be the factor of changing and decreasing the protein level on the non target animals like fishes. Proteins play a vital role in the interaction process of the cellular medium in the organism like *Channa punctata* (Magar and Shaikh, 2012). Tiwari (2004) observed that sub-lethal concentrations of malathion for different time exposure showed a significant decrease in total, structural and soluble protein content in liver tissue of *Cirrhinus mrigala* showing similarity to the present study. Jha and Verma (2002) exposed *Clarius batrachus* to the sub-lethal doses of pesticidal mixture (Endosulfan; Malathion and Agrafun 1:1:1) and observed similar response of reduced protein profile in various tissues under acute (96 hours), sub chronic (7 days and 14 days) and chronic (21 days) exposure in dose and time dependant manner. Similar trend of decreasing total protein level was also observed in *Channa punctata* (Tilak *et al.*, 2003) and *Catla catla* (Anita Susan *et al.*, 1999) exposed to sub lethal concentration of fenvalerate. Exposure of *Clarius batrachus* to dimethoate showed decrease in protein content indicating physiological adaptability of the exposed fishes to compensate for toxic stress (Ghousia *et al.*, 1995). The present study was also consistent with the findings of Sastry and Siddiqui (1984) who reported decreased protein content in liver, muscle, kidney, intestine, brain and gill of quinalphos exposed *Channa punctatus*. Decrease in protein content as reported by Choudhary and Gaur (2001) in *Cyprinus carpio*, Tripathi and Singh (2003) in freshwater snail *Lymnaea acuminate*, Kumar *et al.*, (2004) in *Anabus testudineus*, Muley *et al.*, (2007) in *Labeo rohita* also support the present data.

5. Conclusion:

The present piece of work proved that the freshwater fish *Channa punctata* is an excellent test organism under laboratory conditions, as being a responsive species under toxic environment. Under here-adopted experimental conditions and acute exposure, pendimethalin caused significant alterations in the protein content in dose and time dependant manner reflecting degradation of protein and lowered working capacity which may be due to more herbicidal stress. Therefore this parameter can be used as a useful biomarker in assessing herbicide toxicity in aquatic environment.

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