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RESEARCH ARTICLE

Effect of Dichlorvos on hepato-renal functions of Indian catfish *Heteropneustes fossilis* (Bloch)

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Abstract

Present study was carried out to investigate the effect of organophosphate pesticide Dichlorvos (76% EC) at their sub-lethal level on Serum Ammonia, Serum Urea, activity of SGOT and SGPT of the catfish *Heteropneustes fossilis* (Bloch) to assess the hepato-renal function after 10 days, 20 days and 30 days exposure time to Dichlorvos. The 96h LC_{50} value of dichlorvos was calculated by using probit analysis (Finney, 1964) and LC_{50} for 96 hours is found to be 19 ppm. Sub-lethal concentration of 2.5 ppm and 5 ppm were prepared by using standard technique (APHA, 1985). For this study, acclimatized fishes of *Control Group* were being freed from the treatment of Dichlorvos where as acclimatized fishes of *Experimental Groups* were treated with sub-lethal dose of 2.5 ppm and 5 ppm Dichlorvos. It was observed that in all the biochemical parameters, there are significant dose dependent alterations as well as duration dependent alterations.

INTRODUCTION

Pesticides are a very important group of environmental pollutants used in intensive agriculture practices for protection against diseases and pests (Eleršek & Filipič, 2011). Pesticides wherever applied, they found their way into water bodies ultimately affecting aquatic fauna in general and fish in particular. Since the removal of Organochlorine insecticides from use, Organophosphorus compounds or Organophosphates (OPs) have become the most widely used insecticides over the past 60 years for protecting crops, livestock, human health and as warfare agents (Gupta, 2006).

All forms of OPs share the common mechanism of exerting their neurotoxicity by binding to and phosphorylating the enzyme acetylcholinesterase in both the CNS and PNS (Mileson *et al*., 1998).

Pesticides are also well known for causing more toxic effects in teleost fishes (Muthukumaravel *et al.,* 2013). The magnitude of pesticide pollution was studied in the Indian fishes by various workers (Dalela *et al*., 1979; Dubale and Shah, 1984; Shukla and Pandey, 1980; Rashatwar and Ilyas,1984; Sadhu and Mukhopadhyay, 1985; Ghosh and Chatterjee, 1989; Dutta *et al*., 1992; Medda *et al.*, 1995; Shukla et al., 2005).

Dichlorvos is an insecticide of the organophosphate (OP) group. It has been in use since about 1955 and is used in the UK both professionally and in homes and gardens in a number of areas: in agriculture and horticulture (Lakshmanan *et al*., 2013). Dichlorvos is recommended for application as a high or a low volume spray on crops such as paddy, wheat, soyabean, apple, sugarcane, mustard, sunflower and cashew. It is classified by the WHO as a Class IB ‘highly hazardous’ chemical (Das, 2013). The EPA has classified dichlorvos as toxicity class I - highly toxic, because it may cause cancer and there is only a small margin of safety for other effects. Therefore it was decided to carry out an empirical study on the effect of Dichlorvos on hepato-renal functions of Indian catfish *Heteropneustes fossilis* (Bloch).

1. MATERIALS AND METHODS

1.1. **Specimen**: Healthy and sexually mature specimen of *Heteropneustes fossilis* of equal size group (12 ± 3 cm) and average weight (12 to 15 gm) were procured from the local market and the fishes were kept for 15 days in glass aquarium containing 80 litres of fresh water in the laboratory at about water temperature 25 ± 3º c for acclimatization. Fishes were starved for 24 hours prior to the experiment and are not fed during the period of experiment (Dalela *et al.*, 1979).

1.2. **Pesticide**: The organophosphorus pesticide Dichlorvos (76% E.C) was procured from the local market for present investigation purpose.

1.3. **LC_{50} Calculation**: A pilot experiment was done to find out the LC_{50} value of Dichlorvos by probit analysis (Finney, 1964) and LC_{50} for 96 hours is found to be 19 ppm. Sub-lethal concentration of 2.5 ppm and 5 ppm was prepared by using standard technique (APHA, 1985).

1.4. **Plan of the experiment**: In this experiment, the specimens were kept mainly in two groups namely *Control Group* and *Experimental Groups*. *Control Group* is being freed from the treatment of Dichlorvos and the *Experimental Group 1 & 2* is treated with pesticide Dichlorvos of sub-lethal concentration of 2.5 ppm and 5 ppm. Blood samples were collected from the fishes of both the groups at three different time interval (10 days, 20 days and 30 days) and serum was separated by centrifugation technique.

1.5. **Method of Estimation of ammonia and urea:**. Ammonia in Serum was estimated by Modified Fujii-Okuda Method (1966) at the wavelength of 630 nm in spectrophotometer. Urea in Serum was estimated through Modified Berthelot Method by Fawcett and Scott (1960) at the wave length of 570 nm in spectrophotometer at room temperature of 37^0 C with light path 1 cm.

1.6. **Method of determination of SGOT and SGPT activity**: GOT and GPT in Serum was determined by the method of Reitman and Frankel (1957) at the wavelength of 505 nm in spectrophotometer at room temperature of 37^0 C with light path of 1 cm.

2. RESULTS

Findings of the experiment related to the exposure of sub-lethal Dichlorvos to the catfish *Heteropneustes fossilis* (Bloch) are given below;

2.1. **Tables:**

Table 1: Presenting the % deviation of serum ammonia and urea in *Experimental Group 1* from the mean values of *Control Group* (mg/dl) in *Heteropneustes fossilis* (Bloch).

% deviation	10 days	20 days	30 days
Ammonia	48.77	56.56	63.19
Urea	77.56	76.04	26.81

Table 2: Presenting the % deviation of serum ammonia and urea in *Experimental Group 2* from the mean values of *Control Group* (mg/dl) in *Heteropneustes fossilis* (Bloch).

% deviation	10 days	20 days	30 days
Ammonia	65.03	74.29	73.63
Urea	83.6	83.13	30.53

2.2. **Serum Ammonia:** In comparison to the mean values of serum ammonia in the *Control Group* which is higher than any *Experimental Group*, mean values of the serum ammonia in *Experimental Group 1& 2* were recorded to be in decreasing trend with the increase of exposure time and the sub-lethal concentration of Dichlorvos (Chart 1).

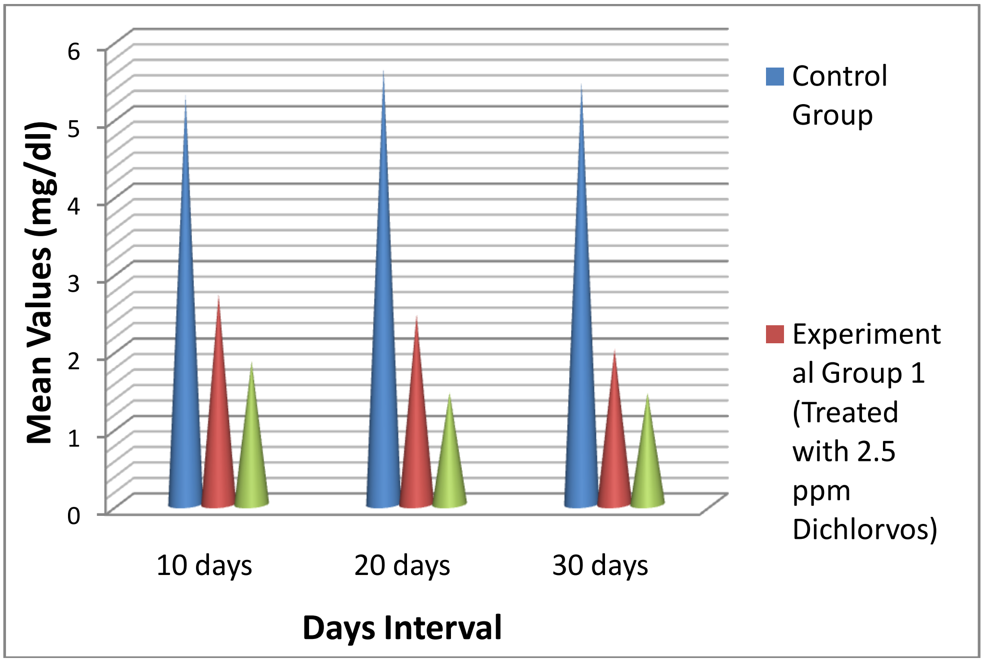

Chart 1: Depicting the mean values of serum ammonia (mg/dl) in *Control* and *Experimental Group1 & 2* of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of Dichlorvos.

2.3. Serum Urea: Mean values of the serum urea were found to be increased in *Experimental Group 1& 2* with the increase of exposure time and the sub-lethal concentration of Dichlorvos but mean values in *Control Group* were observed to be lower than *Experimental Group 1& 2* (Chart 2)

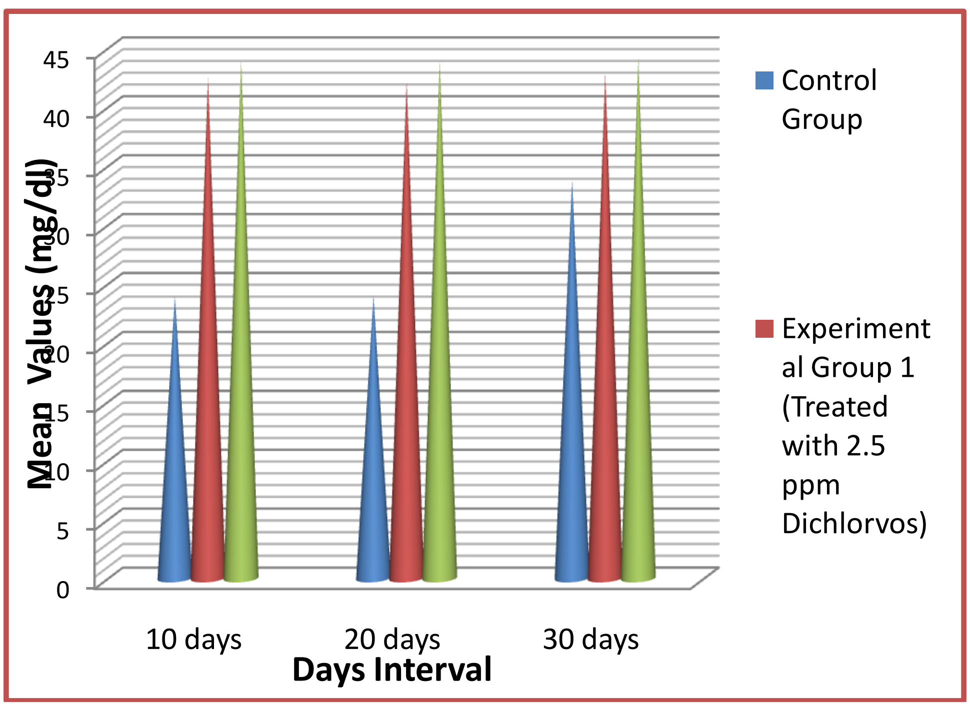

Chart 2: Depicting the mean values of serum urea (mg/dl) in *Control* and *Experimental Group1 & 2* of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of Dichlorvos.

2.4. **SGOT:** Compared to the mean values of the *Control Group*, mean value of the SGOT was observed to be increased steadily in the *Experimental Group 1* with the increase of exposure time of sub-lethal dose of Dichlorvos. Mean value of SGOT in *Experimental Group 2* after 10 days exposure time to Dichlorvos was observed to be the highest among all exposures followed by the mean value of SGOT after 30 days and 20 days exposure time to Dichlorvos. In 20 days exposure time to Dichlorvos, although *Experimental Group 2* was recorded with the lowest mean value of SGOT, but, it was still higher than the *Control Group* (Chart 3).

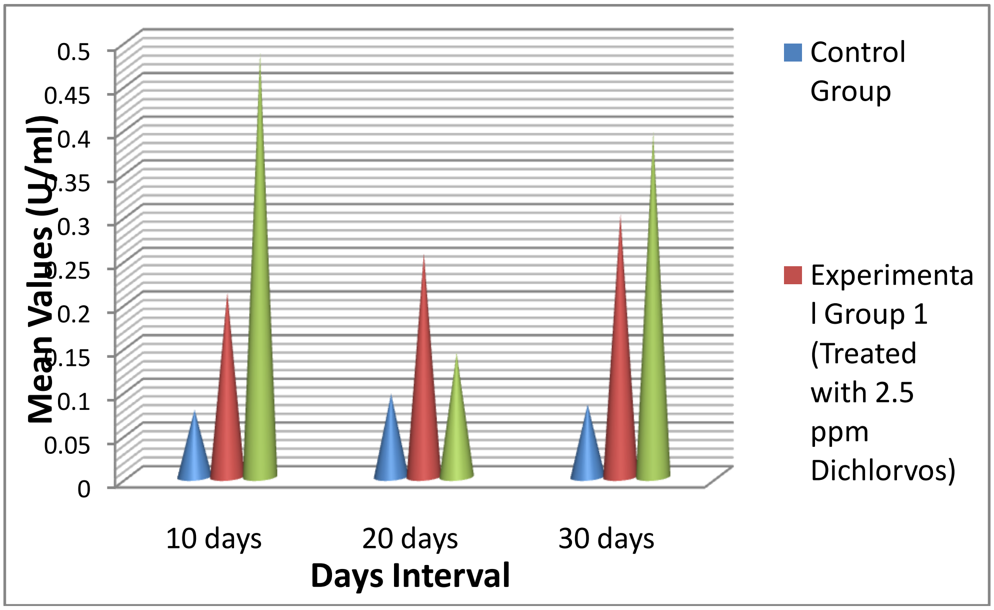

Chart 3: Depicting the mean values of SGOT (U/ml) in *Control* and *Experimental Group1 & 2* of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of Dichlorvos.

2.5. **SGPT:** Compared to the mean values of the *Control Group*, mean value of the SGPT was found to be increased steadily in the *Experimental Group 1* with the increase of exposure time of sub-lethal dose of dichlorvos. Mean value of SGPT in *Experimental Group 2* after 20 days exposure time to Dichlorvos was observed to be the highest among all exposures followed by the mean value of SGPT after 10 days and 30 days exposure time to Dichlorvos. In 30 days exposure time to Dichlorvos, although *Experimental Group 2* was recorded with the lowest mean value of SGPT but it was still higher than the *Control Group* (Chart 4).

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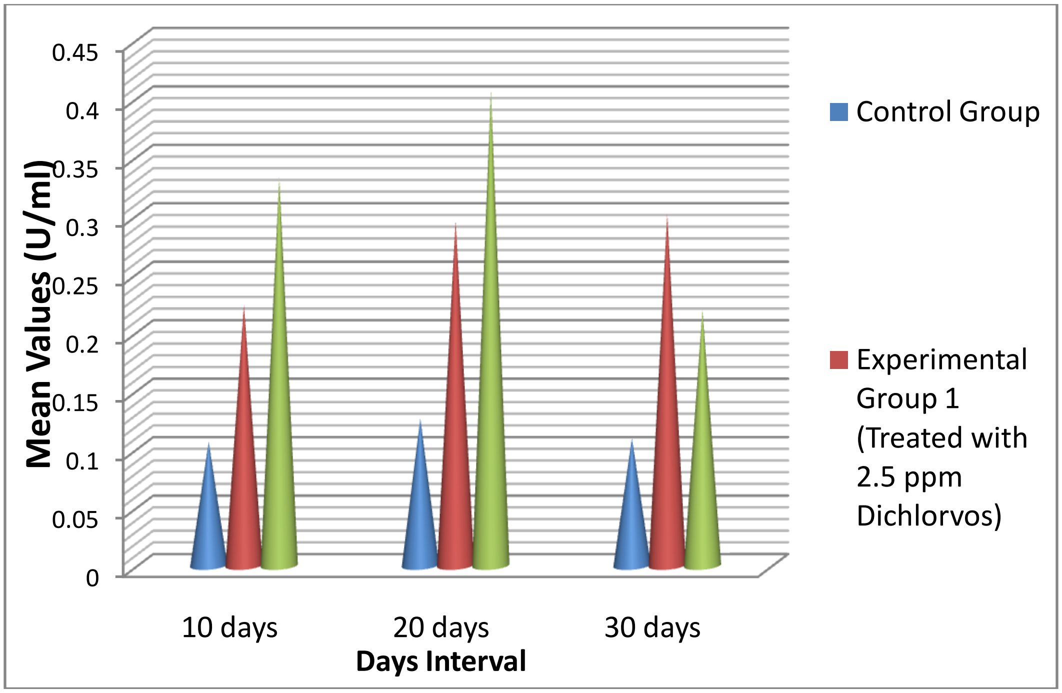

Chart 4: Depicting the mean values of SGPT (U/ml) in *Control* and *Experimental Group1 & 2* of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of Dichlorvos.

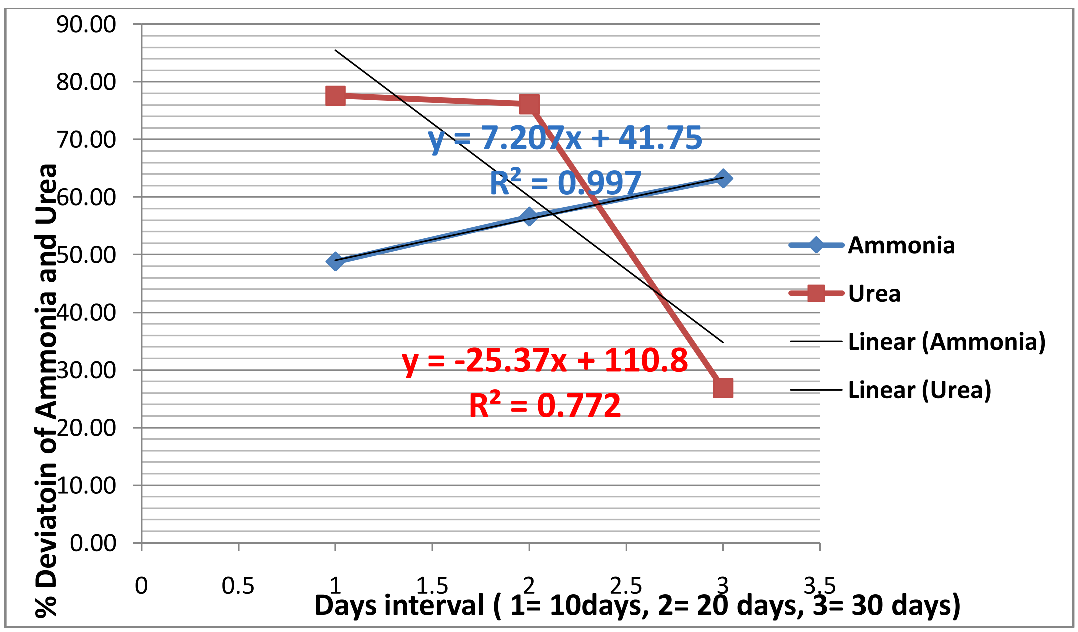

Chart 5: Presenting the correlation scatter graph between the mean values of serum ammonia (mg/dl) and serum urea (mg/dl) in *Experimental Group 1* in *Heteropneustes fossilis* (Bloch).

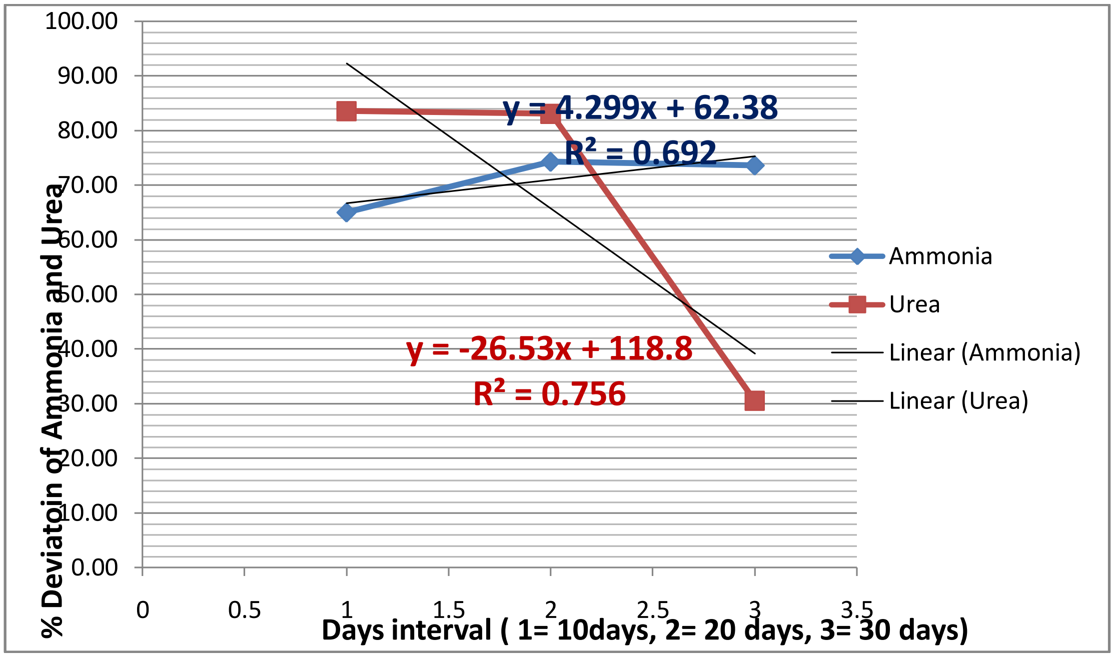

Chart 6: Presenting the correlation scatter graph between the mean values of serum ammonia (mg/dl) and serum urea (mg/dl) in *Experimental Group 2* in *Heteropneustes fossilis* (Bloch).

3. DISCUSSION

From the results it is evident that the catfish *Heteropneustes fossilis* (Bloch) reared in water treated with organophosphate pesticide Dichlorvos produces significant amount of urea in comparison to ammonia through Ornithine Cycle in liver although fishes are mostly ammonotelic in nature. Urea formation occurs in liver in most of the vertebrates. Urea formation is either increase or decrease in liver during any pathological condition of liver. In teleost fish, ammonia excretion accounts for 55 to 80 % of total nitrogen excretion, while urea excretion is about 6 to 8 % (Vellas & Serfaty, 1974). Common cause for increase in blood urea is atypical nitrogen excretion usually due to kidney damage and urinary obstruction (Goel, 1996). However, under some circumstances as stress or enhanced ammonia level in the surrounding, fishes are reported to change their nitrogen excretion mechanism by forming urea as the end product for nitrogen excretion (Saha et al., 2003).

The correlation study between the serum ammonia and serum urea concentrations of *Heteropneustes fossilis* (Bloch) as presented in chart 5 and 6 establishes presence of a definite relationship between these two parameters.

An unstable state of ureotelism is attained with concomitant conversion of ammonia to urea in liver in response to increase in blood ammonia concentration.

Increased activity of the enzyme Glutamate Oxaloacetate Transaminase (GOT, also known as AST) and Glutamate Pyruvate Transaminase (GPT, also known as ALT) in serum is an indication of liver damage in the catfish *Heteropneustes fossilis* (Bloch). Because, transaminases are intracellular enzyme and only a minute quantity is found in the serum. For that reason, damage to the liver cell may cause the enzymes to leak into the plasma/serum due to large concentration gradient (Wroblewski and LA Due, 1955).

4. CONCLUSION

Although an unstable state of ureotelism was observed in this study, yet this study is not enough to draw a conclusion of definite relationship among the parameters. However, determination of transaminase activities in serum of fish can be used as an indicator for toxicant exposure.

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