

EFFECT OF CARBOFURAN ON HAEMATOLOGICAL PARAMETERS OF *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

The present study was aimed to investigate the effect of carbofuran on haematological parameters of *Channa punctatus* (Bloch) was investigated. Carbofuran produced detrimental effects on haematological parameters of *C. punctatus* after 12, 24, 48 and 96 h exposure to 1, 5 and 10 ppm of the insecticide. The haemoglobin content was 85.0 ± 00 % in control group that was gradually reduced to 66.0 ± 5.28% (p=0.011, p<0.05) at 10 ppm. The result also revealed significant reduction in the number of leucocytes (control 15.0 ± 00, treated group 8.75 ± 0.85, p=0.0006, p<0.001), lymphocytes (control group 55 ± 00, treated group 48.75 ± 1.75, p=0.013, p<0.05), basophils (control group 3.0 ± 00, treated group 2.5 ± 0.29, p=0.097, p<0.1), monocytes (control group 5.0 ± 00, treated group 2.50 ± 0.65, p=0.008, p<0.001) and thrombocytes (control group 50.0 ± 00, treated group 39.75 ± 1.65, p=0.0008, p<0.001). However the number of eosinophils (control group 5.0 ± 00, treated group 6.0 ± 0.41, p=0.16, p>0.1) and neutrophil (control group 31.0 ± 00, treated group 39.75 ± 1.75, p=0.0025, p<0.005) were increased in treated group comparing to that of control group.

The ESR (mm/hr) values increased in treated groups as 7.9 ± 1.3 (p>0.1) 7.98 ± 1.28 (p>0.1) and 8.25 ± 1.35 (p>0.1) from 5.5 ± 0.50 of control groups by 1, 5 and 10 ppm exposures respectively.

Keywords: Effect; Carbofuran; *Channa punctatus*; Haematological parameter

INTRODUCTION

The natural environment is contaminated with different kinds of anthropogenic pollutants (Jemal *et al.*, 2002). Insecticides are considered one of the anthropogenic pollutants for the aquatic environment. They in general, are used very extensively in agriculture, forestry, public health and in veterinary practice. Insecticides are useful tools in agriculture and forestry but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Basak and Konar, 1977). A survey by the Bangladesh Fisheries Research Institute reported that 34% of farmers applied insecticides in over doses and 76 % of them have no idea for application of insecticides properly (FRI, 1991). Insecticides can pollute water by direct application in aquatic ecosystem. Indirect uses such as agricultural waste water infiltration which eventually washed into deep water environment and ecosystem also cause pollution (Dutta and Arends, 2003). Indiscriminate use of insecticides on crop fields causes serious environmental hazards affecting aquatic animals. Among them, fishes are one of the most vulnerable items. These insecticides may enter into the natural water bodies destroying spawning grounds and feeding area as well as reduce resistance to diseases which can hamper fish

production (Metelev *et al.*, 1983). Now days, insecticides are the main cause of toxicity in fish. *Channa punctatus* is one of the most popular fish in Bangladesh and is now one of the most endangered fish species too.

Extensive use of insecticides by farmers in the crop fields may cause the species (*C. punctatus*) much endangered (Hossain *et al.*, 2000). Carbofuran is a granular insecticide of carbamate group (Hydrolysis half-life in water 5.1 weeks at p^H 7.0 and 1.2 h at p^H 10) which may produce serious damage to fish health ((HSDB, 1999)). Carbofuran may kill a wide range of non-target species and is extremely toxic to aquatic organisms including fishes. Haematological effects in fish due to various toxicants have been reported by several investigators (Strik *et al.*, 1976; Lunn *et al.*, 1976; Pandey *et al.*, 1979; Mahajan and Dheer, 1979; and Panigrahi, 1977). Haematological parameters reflect the state of fish under stress more quickly than other commonly measured parameters as they respond quickly to changes in environmental conditions (Alkinson and Jurd, 1978). For monitoring stress responses and physiological adaptation of animals, haematological parameters have been widely used for the description of general health of fish (Seriani *et al.*, 2012).

Erythrocytes, leucocytes and thrombocytes are the essential cellular components of fish blood. Blood parameters are considered as pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicity of carbofuran (Adhikari *et al.*, 2004). A number of haematological indices such as haemoglobin, red blood cells (RBC), oxygen carrying capacity of the blood stream have been used as indicators of metal pollutants in the aquatic environment (Shah and Altindag, 2004). Lymphocytes of fish have been regarded as immunocompetent and thus, they are responsible for the production of antibodies as a result immunological reaction (Ellis *et al.*, 1978). White blood cells play a major role in defense mechanism and mainly comprise granulocytes, monocytes and lymphocytes (Jurd, 1985). When lymphocyte and monocyte can be affected the immune system of the fish affected. Thus the fish become weak and may be easily infected by different types of pathogens. *C. punctatus* is now one of the rare species in Bangladesh floodplains. It is assumed that indiscriminate use of carbofuran may cause the species (*C. punctatus*) very rare in the floodplains of Bangladesh. Therefore the objective of the present study was to investigate the haematological changes in *C. punctatus* when exposed to different concentrations of carbofuran.

MATERIALS AND METHODS

Experimental fish

A total of 40 live *Channa punctatus* weighing 37.58 ± 0.79 g and measuring 15.01 ± 0.27 cm were collected from a fish landing centre of Rajshahi. The experimental fishes were transported to the laboratory and kept in 60 L tank with natural pond water. The fishes were acclimatized to laboratory conditions for one week at $26 \pm 0.1^\circ\text{C}$. The same temperature was maintained throughout the experiment. The water in aquaria was properly aerated to avoid any contamination. Test glass aquaria were used for conducting experiment. The glass aquaria were washed with detergents and 0.1% potassium permanganate and dried under sunlight. Each aquarium was filled with 3 liters of pond water with 10 fishes. A control group of fish also reared throughout the experiment.

Dose exposure of experimental fish

The experimental fishes were treated with different concentrations, *viz.* 0 (control) 1, 5 and 10 ppm of carbofuran for 12, 24, 48 and 96 h. Fishes were fed with commercial feed pellets (Osaka, Perfect Companion Group Co., Ltd. Samutprakarn 10540 Thailand) twice a day until completion of experiment.

Blood collection

Fishes are anesthetized by using anesthetic agent like MS-222 (tricaine methanesulfonate). Blood samples were collected by caudal puncture for the determination of haemoglobin, leucocytes, lymphocytes, monocytes,

basophils, neutrophils, eosinophils, thrombocytes and ESR. Blood sample was also collected from control group.

Estimation of leucocyte count and its differentials

For the estimation of leucocyte and its differentials, haematological slides were prepared and the number of leucocytes was calculated ($\text{mm}^3 \times 10^3$) (Wintrobe, 1967). Differentials like lymphocytes, monocytes, eosinophils, basophils, thrombocytes and neutrophils were also counted according to Dacie and Lewis, 1977.

Estimation of haemoglobin

A sample of 20 μl blood was collected from experimental fishes by caudal puncture and put into graduated haemoglobin tube containing EDTA as anticoagulant. Then the estimation of haemoglobin content was done (Van and Zijlstra, 1961). Statistical analysis was performed to compare the differences between the values of treated groups and control group.

Statistical analysis

Descriptive statistics was used to analyze the data of interest.

Estimation of erythrocyte sedimentation rate (ESR)

The fishes were exposed to 0 (control), 1, 5 and 10 ppm of carbofuran for 6 and 12 h. After 6 and 12 h exposure to 1, 5 and 10 ppm concentrations of carbofuran they were sacrificed for blood collection from which a sample of 1.6 ml blood for each fish was collected. Then the blood was mingled with 0.4 ml of ESR fluids. A total of 2 ml samples (both fish blood and ESR fluid) filled up to zero limit of ESR stand. The sedimentation rate was taken vertically from ESR stand in mm/hr.

RESULTS

Haematological changes due to carbofuran application

The haematological changes that occurred in *C. punctatus* due to the application of carbofuran were recorded. A significant reduction in the number of haemoglobin ($p=0.011$), leucocytes ($p=0.0006$), lymphocytes ($p=0.013$), monocytes ($p=0.008$), basophils ($p=0.097$) and thrombocytes ($p=0.0008$) except for eosinophils ($p=0.16$) and neutrophils ($p=0.0025$) at 1, 5 and 10 ppm of carbofuran after 12, 24, 48 and 96 h exposures.

Haemoglobin (%)

The average haemoglobin count was recorded as 85.0 ± 00 in control fish. It was plunged and recorded as 76.0 ± 3.24 ($p<0.05$), 70.25 ± 3.97 ($p<0.01$) and 66.0 ± 5.28 ($p<0.05$) after 12, 24, 48 and 96 h exposed to 1, 5 and 10 ppm respectively in treated fish (Table 1 and Figure 1).

Leucocytes ($10^3/\text{mm}^3$)

The average leukocyte count was recorded as 15.0 ± 00 in control fish, whereas a dose dependent significant reduction in the number of leucocytes was recorded 12.5 ± 1.04 , 10.75 ± 0.85 and 8.75 ± 0.85 after 12, 24, 48 and

| Parameters | Control fish | Exposed fish | | |
|--|--------------|---------------------------|---------------------------|---------------------------|
| | | 1 ppm | 5 ppm | 10 ppm |
| Haemoglobin (%) | 85.0 ± 00 | 76.0 ± 3.24 ^a | 70.25 ± 3.97 ^c | 66.0 ± 5.28 ^a |
| Leucocytes (10 ³ /mm ³) | 15.0 ± 00 | 12.50 ± 1.04 ^c | 10.75 ± 0.85 ^c | 8.75 ± 0.85 ^c |
| Lymphocytes (%) | 55.0 ± 00 | 50.50 ± 1.44 ^a | 50.0 ± 1.47 ^a | 48.75 ± 1.75 ^a |
| Monocytes (%) | 5.0 ± 00 | 4.0 ± 0.41 ^a | 3.0 ± 0.41 ^c | 2.50 ± 0.65 ^b |
| Eosinophils (%) | 5.0 ± 00 | 6.50 ± 0.65 ^d | 7.0 ± 0.41 ^b | 6.0 ± 0.41 ^d |
| Basophils (%) | 3.0 ± 00 | 3.50 ± 0.29 ^d | 3.0 ± 0.41 ^d | 2.50 ± 0.29 ^b |
| Neutrophils (%) | 31.0 ± 00 | 35.50 ± 1.04 ^b | 37.0 ± 1.58 ^a | 39.75 ± 1.75 ^b |
| Thrombocytes (10 ³ /mm ³) | 50.0 ± 00 | 42.75 ± 1.25 ^b | 40.75 ± 1.25 ^c | 39.75 ± 1.65 ^b |

Each value represents the mean ± SE of four replications of 12, 24, 48 and 96 h exposure time (a, b & c refers to the Level of significance ^a*p*<0.05, ^b*p*<0.01 and ^c*p*<0.001 and d indicates insignificant *p* values, *p*>0.1).

Table 1: Effect of carbofuran on some haematological parameters of *Channa punctatus* at 1, 5 and 10 ppm after 12, 24, 48 and 96 h of exposures.

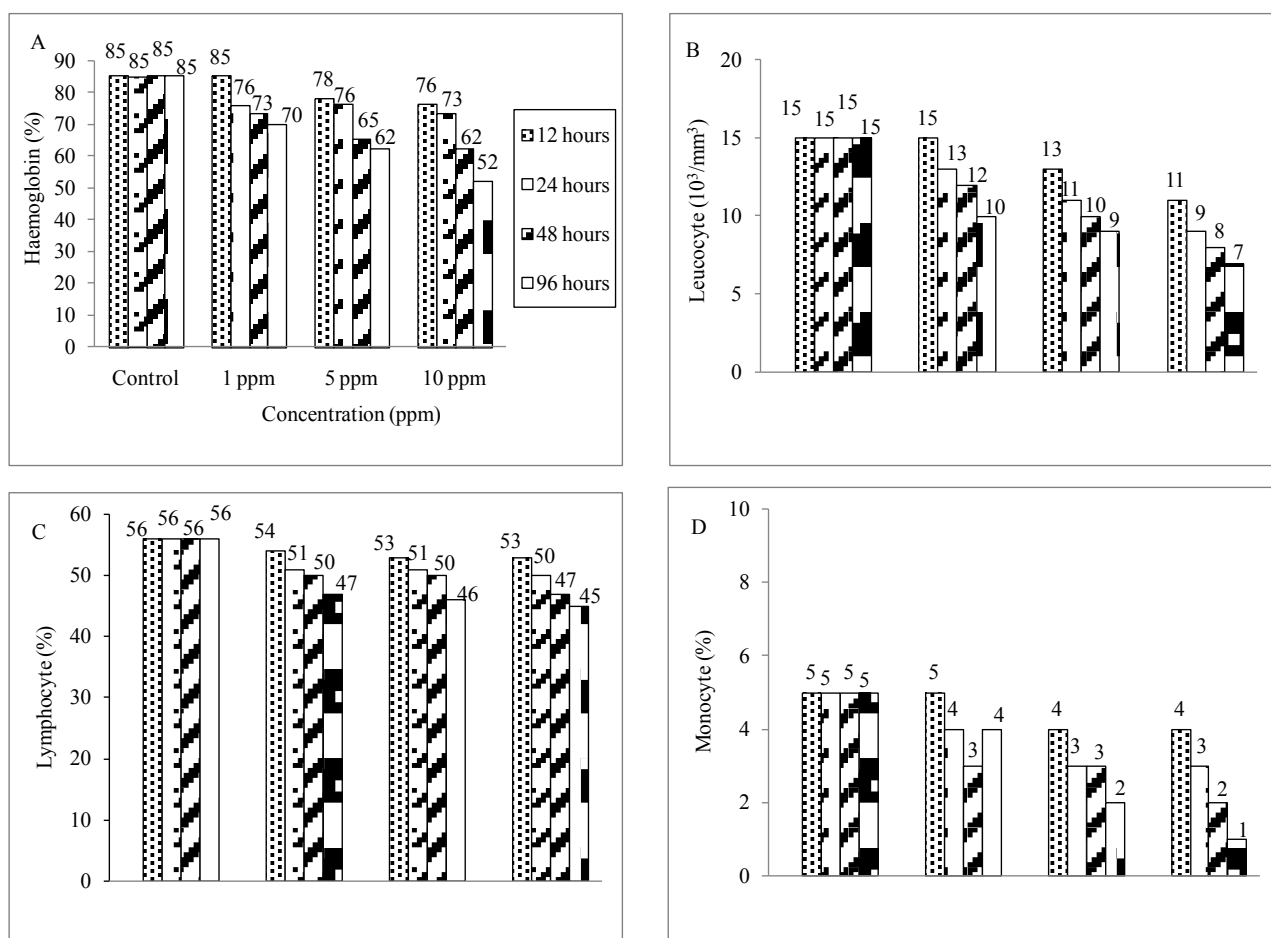


Figure 1: Percentages of haemoglobin (A), leucocytes (B), lymphocytes (C) and monocytes (D) during exposure periods of 12, 24, 48 and 96 h at 1, 5 and 10 ppm of carbofuran.

96 h exposure to 1, 5 and 10 ppm respectively (*p*<0.01) in treated fish (Table 1 and Figure 1).

Lymphocytes (%)

The number of lymphocyte in control fish was an average of 55.0 ± 00. But the lymphocyte count was declined to the average values of 50.50 ± 1.44, 50.0 ± 1.47 and 48.75 ± 1.75 after 12, 24, 48 and 96 h exposures to 1, 5 and 10 ppm respectively (Table 1 and Figure 1), (*p*<0.05).

Monocytes (%)

The average monocyte count in control fish was

estimated 5.0 ± 00. On the contrary, in treated fish, it was went down and counted 4.0 ± 0.41 (*p*<0.05), 3.0 ± 0.41 (*p*<0.01) and 2.50 ± 0.65 (*p*<0.01) after 12, 24, 48 and 96 h exposed to 1, 5 and 10 ppm respectively (Table 1 and Figure 1).

Eosinophils (%)

The average eosinophil count was recorded as 5.0 ± 00 in control fish. In case of treated fish, the number of eosinophil was increased and estimated as 6.5 ± 0.65 (*p*>0.1), 7.0 ± 0.41 (*p*<0.05) and 6.0 ± 0.41 (*p*>0.1) after 12, 24, 48 and 96h exposed to 1, 5 and 10 ppm respectively (Table 1 and

Figure 2). At 1 and 10 ppm, the data showed statistically insignificant values.

Basophils (%)

An average number of basophil in control fish was recorded 3.0 ± 0.0 . But in treated fish, it was observed 3.5 ± 0.29 at 1 ppm, 3.0 ± 0.41 at 5 ppm and 2.5 ± 0.29 ($p < 0.01$) at 10 ppm after 12, 24, 48 and 96 h exposures (Table 1 and Figure 2) and the data finally turned down with statistically insignificant p values at 1 and 5 ppm ($p > 0.1$).

Neutrophils (%)

Neutrophil content in control fish showed 31.0 ± 0.0 . On other hand, a significant increase in neutrophil content was inflated significantly and recorded 35.50 ± 1.04 ($p < 0.01$) at 1 ppm, 37.0 ± 1.58 ($p < 0.05$) at 5 ppm and 39.75 ± 1.75 ($p < 0.1$) at 10 ppm after 12, 24, 48 and 96 h exposures to carbofuran (Table 1 and Figure 2).

Thrombocytes ($10^3/\text{mm}^3$)

In control fish, the average number of thrombocytes was as recorded 50.0 ± 0.0 . A significant dose dependent reduction in the number of thrombocytes in treated fish was recorded 42.75 ± 1.25 , 40.75 ± 1.25 and 39.75 ± 1.65 after 12, 24, 48 and 96 h exposure to 1, 5 and 10 ppm respectively when compared to the value of control group (Table 1 and Figure 2) ($p < 0.01$).

ESR (Erythrocyte Sedimentation Rate, mm/hr)

The ESR value (mm/hr) in case of control fish was an average of 5.50 ± 0.5 mm/hr. An increase in the ESR value was recorded 7.9 ± 1.3 at 1 ppm, 7.98 ± 1.28 at 5 ppm and 8.25 ± 1.35 at 10 ppm of carbofuran after 6 and 12 h exposures respectively with comparing to that of control group (Table 2) ($p > 0.1$).

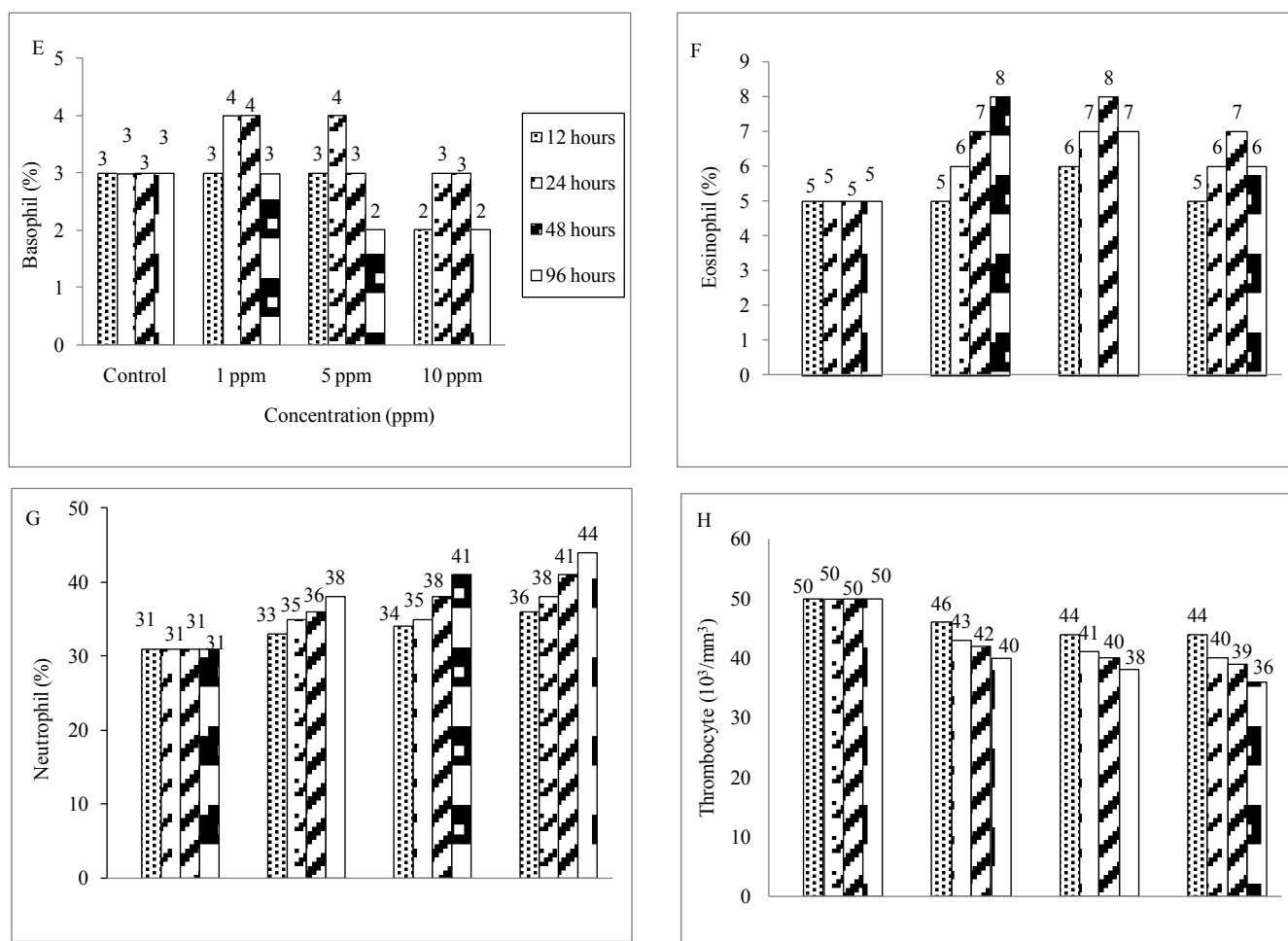


Figure 2: Percentages of basophils (E), eosinophils (F), neutrophils (G) and thrombocytes (H) during exposure periods of 12, 24, 48 and 96 h at 1, 5 and 10 ppm of carbofuran.

| Trial | Exposure period (hour) | Control fish | Treated fish | | |
|-----------|------------------------|--------------|--------------|-------------|-------------|
| | | | 1 ppm | 5 ppm | 10 ppm |
| 1 | 6 hour | 5.0 | 6.6 | 6.7 | 6.9 |
| 2 | 12 hour | 6.0 | 9.2 | 9.25 | 9.6 |
| Mean ± SE | | 5.50 ± 0.5 | 7.9 ± 1.30 | 7.98 ± 1.28 | 8.25 ± 1.35 |

Table 2: Effect of Carbofuran on ESR (mm/hr).

DISCUSSION

Haematological parameters of fishes constitute an important biological system for their survival against diseases. Insecticidal contamination to aquatic ecosystem can affect haematological parameters of fish (Summarwar, 2012). It was reported that carbofuran caused the reduction in the value of hemoglobin in teleost fishes (Singh and Srivastava, 2010). A significant decrease in the haemoglobin content was observed when the experimental fish (rain-bow trout) was exposed to diazinon (Far *et al.*, 2012). A significant decrease in haemoglobin content was recorded in *Cyprinus carpio* (Adedeji *et al.*, 2009). In the Nile Tilapia (*Oreochromis niloticus*) a marked decrease in haemoglobin content was also observed due to exposure to dimethoate and malathion in the same species (Sweilum, 2006).

A reduction in leucocyte count was observed in *C. punctatus* after chronic exposure of freshwater teleosts to monrotophos (Singh *et al.*, 1992). A significant decrease in leucocyte count due to the exposure of *Cyprinus carpio* to toxic environment of diazinon (Banaee *et al.*, 2008) was reported. However, a significant rise in leucocyte content was reported in *C. punctatus* due to toxic effects of malathion (Magar and Duve, 2012).

A significant decline in lymphocyte count and a marked increase in neutrophil in *C. carpio* when exposed to diazinon has been recorded (Velisek *et al.*, 2009). The number of lymphocyte was significantly decreased and neutrophil was increased was observed (Saeedi and Singh, 2013). An increase in the number of neutrophils has been reported in fresh water fishes treated with fenvalerate and malathion (Mukhupadhay and Dehadaria, 1980). A significant increase in neutrophil was also observed due to toxic stress induced by the pulp mill effluent (Thakur and Pandey, 1990). Monocrotophos decreased neutrophil count whereas increased the number of lymphocytes in freshwater fish, *Channa punctatus* (Agrahari *et al.*, 2006). The reductions in lymphocyte and rise in neutrophil content could be due to the destruction of haematopoietic tissue and decrease in non-specific immune system due to increased concentration of defensive poison.

A study was conducted to estimate the haematological parameters of *C. punctatus* when exposed to Rayon industry effluents. The effluents caused reduction in monocyte content in *C. punctatus* (Banerjee *et al.*, 2003). A marked decline in monocytes was observed when *H. fossilis* was exposed to lindane (Srivastava and Mishra, 1985). The effects of monocrotophos on erythropoietic activity and haematological parameters of *C. punctatus* with a reduction in monocyte count were observed (Agarwal *et al.*, 2006). Sublethal concentrations of synthetic pesticide, dimethoate exhibited a significant rise in the percentage of monocytes in *H. fossilis* (Ghosh and Banerjee, 1993). The reduction in the number of monocytes in present study may be due to toxic effects of carbofuran on the kidney and spleen (haematopoietic tissues).

An increase in eosinophil content in *H. fossilis* due to malathion has been recorded (Mishra and Srivastava, 1983). Toxicity of diazinon enhanced the number of eosinophils in *Cyprinus carpio* (Svobodova *et al.*, 2001). A reduction in eosinophils when the experimental fish was exposed to malathion was observed (Metelyev and Grischenki, 1970). The number of eosinophils was increased while basophils were decreased after 60 days exposure of *C. batrachus* to carbon tetrachloride (Sharma and Gupta, 1982). The maximum decrease in percentage of basophils was recorded after 45 days of exposure (Jayaprakash, 2013).

Acute and chronic exposure to *Clarias batrachus* with sublethal concentrations of endosulfan showed a decreasing trend in thrombocyte count along with retarded blood coagulation time (Gopal *et al.*, 1982). A marked decline in thrombocyte count was found in *Heteropneustes fossilis* due to heptachlor treatment (Shrivastava and Mirsha, 1987). Endosulfan treatment of *C. batrachus* caused a significant increase in circulating thrombocytes (Venkateswarlu *et al.*, 1990). In the present investigation a marked decline in thrombocyte count due to carbofuran toxicity, may be due to impaired haemopoiesis and disintegration of thrombocytes in the form of capillary haemorrhage.

An increase in ESR (mm/hr) has been reported in *C. batrachus* after exposure to savin (Kumar and Benergee, 1990) and in *Heteropneustes fossilis* when exposed to alachlor and royor (Chaturvedi and Agarwal, 1993). The present study reveals that ESR is negatively correlated with total erythrocyte count *i.e.* lower the erythrocyte count higher will be the ESR. Carbofuran toxicity may disrupt erythropoietic activity in *C. punctatus*. Above all, a sublethal concentrations of Carbofuran enhanced the number of leucocytes, lymphocytes, monocytes and neutrophils and dropped the content of haemoglobin in the fresh water fish, *C. punctatus* (Shahi *et al.*, 2013).

However, it was also reported by Sign and Srivastava (2010) that the contents of haemoglobin, leucocytes, lymphocytes and thrombocytes were reduced whereas neutrophils, eosinophils and ESR contents were increased due to the effects of carbofuran and carbaryl.

Our results are in close conformity with those reported above. However, more comprehensive works are to be solicited.

CONCLUSIONS

The detrimental effects of carbofuran are very much reflected in the significant reduction in various haematological parameters of *C. punctatus*. This suggests that the environment must be protected against any toxicity produced by chemical agents.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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