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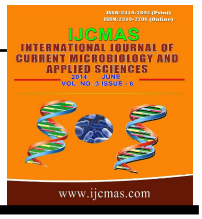

Original Research Article

Toxicity of sub-lethal concentrations of Monocrotophos (MCP) on the haematological, biochemical and growth responses of hybrid catfish, Heteroclarias and contaminated-Heteroclarias fed rats

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A B S T R A C T

Toxicity of sub-lethal concentrations of Monocrotophos (MCP) on the haematological, biochemical and growth responses of hybrid catfish *Heteroclarias* and contaminated *Heteroclarias*-fed rats were investigated. *Heteroclarias* were assessed in a static renewal bioassay for 28 days using varying concentrations (0.00µg/l, 0.15µg/l, 0.20µg/l, 0.25µg/l, 0.30µg/l, and 0.35µg/l) of MCP, while the rats were fed with diet compounded with MCP-contaminated fish for 30 days. At the end of the experiments, the fish and the rats were sacrificed and blood samples were collected. The gill and liver of fish as well as the liver and kidney of rats were removed for bioassay. Values of haematological variables such as red blood cell counts (RBC), haemoglobin (HB), haematocrit (PCV) and glucose showed a significant reduction ($P < 0.05$) compared to the control in both fish and rats. While the values of white blood cell (WBC) and protein increased insignificantly ($P > 0.05$), the mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) significantly decreased ($P < 0.05$) when compared to the control in fish; but MCH decreased insignificantly ($P > 0.05$) and MCHC increased insignificantly ($P > 0.05$) in rats. Compared with the control, the result showed a significant increase ($P < 0.05$) in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), lactate dehydrogenase (LDH) and creatinine in the blood, gill and liver of exposed fish as well as in the blood, liver and kidney of exposed rats. The specific growth rate of the fish and the rats decreased significantly ($P < 0.05$) as the concentration increased. This study shows that sub-lethal concentrations of monocrotophos are harmful to *Heteroclarias* and rats. The implication of these results in rational exploitation and conservation of fishery resources and the public health risk of consuming MCP-exposed fish are highlighted.

K e y w o r d s

Monocrotophos, Toxicology, Haematology, Biochemistry, Fish conservation, Public health.

Introduction

Fisheries provide an important source of food, employment, income, and recreation for people throughout the world (Allison, 2001). Appreciations of fisheries and aquatic ecosystems have been accompanied by increasing concern about the effects of growing human populations and activity on aquatic life and water quality. (lious, *et al*., 1996). Out of the many links associated to man`s negative impact on

other neighboring environment, pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality (lious, *et al*., 1996). Among the commonest pesticides used by farmers is monochrotophos (MCP), it is an organophosphate insecticide which is widely used on cotton, maize, sorghum, rice, sugarcane, vegetable and tobacco pests (ACGI, 1991). They have their way in to the water body as a result of runoff of the residue which is said to be highly hazardous and slightly harmful to fish. (WHO, 1986) .Water pollution by pesticides is a serious problem in most aquatic fauna and flora and to a considerable extent man (Yaji and Auta, 2007). The realization of the polluting and potential public health effects of pesticides have therefore prompted a number of studies on the toxicity of Monochrotophos (MCP) on fishes. Many works have been carried out on biochemical parameters (Zikic,*et al*.,2001; Martinez and Colus, 2002; Achuba and Osakwe, 2003; Zang, *et al*., 2003; Zang, *et al.,* 2004; Simonato, *et al.,* 2006; Nwamba, *et al*., 2006 and Ugwu, *et al*., 2007; Soufy *et al*., 2007; Shalby, 2008) and on haematological parameters (Bhatia, *et al*., 2004; Kori-Siakpere *et al.,* 2006, 2007; Patnaik and Patra, 2006; Yaji and Auta, 2007; Kori – Siakpeere and Ubogu, 2008; Sumonu and Oyelola 2008;

Bunmi, 2010; Owolabi, 2011) of African cat fish and rats. Amongst these, reports on potential toxic effects of Monocrotophos (MCP) on *Heteroclarias* are scanty. Also, little information is currently available about the toxicity of MCP on man who consumes fish harvested from water bodies polluted by this toxicant.The aim of this study was to investigate the haematological, biochemical, and growth responses of Hybrid catfish, *Heteroclarias* exposed to toxicant, monocrotophos and contaminated Heteroclarias-fed rats as potential biomarkers to assess pollution by this toxicant.

Materials and Methods

Experimental set up (fish)

Juveniles of *Heteroclarias* (average weight 57.9 g ± 20.75) and average lengths 20.1g ± 0.12) were obtained from a commercial fish hatchery in Ilorin, Kwara State, Nigeria and transported in plastic aquaria containing water from the hatchery to the laboratory. The fish were not fed throughout the day due to stress which may prevent easy digestion and cause mortality. Feeding commenced the following day, and they were fed twice daily at 8:00am and 4:00 pm with commercial feed (Copens 2 mm) at 4% of initial body weight (Meyer, *et al.,* 1993).The hatchery water was replaced by chlorine free bore-hole water and the fish were kept in large tank of 1,200 liter capacity at 24^0C and acclimatized to laboratory conditions for 14 days. Prior to experiments, the water was kept oxygen saturated with aerators. Unconsumed feed and faecal wastes were removed and water was renewed every three days to reduce pollution and the risk of disease outbreak and mortality. The physical and the behavioural changes were observed in the fish, by monitoring the swimming and feeding activities (USEPA, 1996), Feeding was stopped 24 hours before the commencement of the experiment (USEPA 1996).

Experimental Fish and treatments

Based on the result of the range finding test carried out by Adesida, 2009. Zero point three milliliter (O.35 ml) of MCP

was introduced into 30 liters of borehole water and mixed thoroughly to make a stock solution of 3.5 µg/L, of this, five different concentrations (0.35 µg/L 0.30 µg/L 0.25 µg/L 0.20 µg/L and o.15 µg/L) were measured and each was introduced into ten liters of water in fifteen aquaria. Fish were randomly divided into six groups of 10 fish each. One group served as control while the remaining five groups were exposed into the five measured concentrations. The experiment was set in triplicate, of which one set served as the real experiment, while the remaining two sets served as the replicates. (Pathirantne, 1999). The fish were fed once in a day with (Copens 3 mm) at 4% of their body weight according to Meyer, *et al.*, (1993).The fish were weighed at the initial stage and their weights were taken every week and on last day of the experiment. The body mass and standard lengths were recorded as W1, W2, W3, W4, and W5. Both physical and behavioural changes in the fishes in each aquarium, with different concentrations were observed and recorded. (Pthirantne, 1999).The aquaria were covered with mosquito-mesh nets to prevent fish escape or predators. Each aquarium containing the fish was cleaned and toxicant was renewed every three days to keep the concentration of the toxicant constant. The test duration lasted for 28days.

Formulation of Diets

At the end of the 28 days experimental period, some of the fishes were oven-dried at 40^0c and used as a source of protein (25%) to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class comprising corn starch (52 %), oil (4%), maize cob (4%), sucrose (10 %), and vitamin/mineral mixture (5%). The food items were mixed together and made into pellets by pelleting machines to feed the albino rats.

Experimental Rats and treatments

Sixty (60) rats (*Rathus norvegicus)* with an average weight of 63.22 ± 5.77 were obtained from the animal holding unit of the Department of Zoology, University of Ilorin, Ilorin, Nigeria. The animals (rats) were acclimated to the house conditions for 14 days prior to study start. After the acclimatation period, the rats were stratified by weight and randomized into dosed and control groups. The animals were grouped into six with each group containing ten rats. Each group with 10 rats was housed per cage. The first group serves as the control and they were fed on the control diet, which was formulated with fishes raised in bore-hole water. Animals in group 2-6 were fed on diet formulated with catfish exposed to the different concentrations of monochrotophos (0.35 µg/l, 0.30 µg/l, 0.25 µg/l, 0.20 µg/l, 0.15 µg/l) respectively. The rats were monitored and the physical and behavioral changes were observed. Body weights were taken weekly. The feeding lasted for a period of thirty (30) days.

Biochemical Assay

The activities of alamine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in the blood and other tissues (gills, liver and kidney) were analyzed using Tietz method (1982) and Reitman's and Frankel methods (1957) respectively. Lactate dehydrogenase (LDH) was analyzed using Deutche Gessellschaft Klinische chemie (DGKC, 1970). Tissues used for SOD analyzed were homogenized using the similar method (woolliams, *et al*., 1983).

Haematological Assay

The following blood parameters:- packed cell volume (PCV), red blood cell count (RBC), white blood cell count , (WBC); haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), creatinine (CREA), glucose contents and protein level were determined. PCV was determined using wintrobe and wester green methods, and the blood was centrifuged for 5 minutes at 12,00 g. the mean value of PCV(%) were measured with a micro haematoctrits reader (Blaxhall and Daisley, 1973). Improved Neubaurer counting chamber (Blaxhall and Daisley, 1973) was used to estimate both the red blood cells (RBC) and the white blood cell (WBC) concentrations. Haemoglobin concentration was estimated using cyanometh haemoglobin method (Drabkin, 1946).

The values of RBC, HB and PCV, obtained were used to calculate the MCV, MCH and MCHC using the formulae given by Dacie and Lewis (2001).
Jaffe method (Perrone, *et a*l., 1992) was used to determine creatinine in the blood plasma. Glucose in serum was determined using Trinder (1969) method (colorimetric blood). Protein level in serum was estimated using "biurret method" (Lowry, *et al*., 1951).

Growth

The specific growth rate was estimated using the following formulae

$$r3 = \frac{\log_e W_2 - \log_e w_1}{t2-t1} \times 100$$ (Pack, 1991)

Where r3 = the specific growth rate
$\log_e w_1$ =Logarithm of average weight of fish in the tank at the start of the study.
$\log_e w_2$ = Logarithm of average weight of fish in the tank at the end of the study.
t1, t2 = time in days at the start and end of the study.

Statistical Analysis

Analysis of variance (ANOVA) and Duncan multiple range tests were used to test for differences between different levels of treatments and to separate means respectively. Test of significance were at 95% ($P < 0.05$) probability (Duncan, 1955).

Results and Discussion

The enzymes activities in the blood, gill and liver of *Heteroclarias* exposed to different concentrations of MCP for 28days are shown in Table 1. The values of ALT and AST decreased significantly in the blood, and in the gill of fish, but increased significantly ($p < 0.05$) in the liver as the concentration of MCP increased. While the values of LDH decreased in the blood significantly ($p<0.05$) but increased in the gill and in the liver of the fish with increase in the concentration, and the values of SOD increased in the blood and in the liver, but decreased in the gill.

The enzymes activities in the blood, kidney and liver of rat fed on diet containing different concentrations of MCP for 30days is shown in Table 2. There was a significant decrease in the values of ALT, AST, LDH, and Creatinine, and an increase in the values of SOD in the blood with increase in the concentration. The values of ALT, AST, and LDH decreased in kidney as the toxicant concentration increased, while that of SOD showed no significant

difference. But the values of ALT, AST, LDH and SOD decrease significantly in the liver of the rat as the concentration increased.

Haematological parameters of *Heteroclarias* exposed to MCP for 28 days in Table 3 showed marked and significant decrease in erythrocytes. After 28 days of exposure, the total RBC counts, HB and PCV decreased significantly ($P < 0.05$) as the concentration increased compared to control value. Values of WBC showed an insignificant increase ($P < 0.05$) as the concentrations increased, but showed decrease in values in the highest concentration (250.00 pm) of MCP. The values of the protein showed insignificant increase with increase in concentration. Haematological indices- MCV, MCH and MCHC value reduces significantly ($P < 0.05$) as compared to control.

Noticeable differences were observed in haematological parameters of exposed rat after 30 days of exposure. Exposed rat showed a significant ($P < 0.05$) decrease in number of erythrocytes (Table 4) as compared to control value. The values of HB and PCV also showed a significant ($P < 0.05$) decrease with increase in concentration with the control group having the highest values and the group exposed to the highest concentration (0.35 µg/l) showed the lowest values. The value of WBC increased with increase in concentration, but the values decreased with the highest concentrations(0.30 µg/l and 0.35 µg/l) and the control recording the lowest value. Haematological indices MCV showed an insignificant decrease while the MCH showed an insignificant increase. The values of MCH and MCHC in the control and the exposed groups' recorded similar superscripts in the same homogenous group thus exert similar effect in the blood.

The mean weight gain and specific growth rate of *Heteroclarias* and albino rats exposed to different concentrations of MCP for 28 days and 30 days respectively are shown in the Tables 5 and 6 , also on figures 1, 2, and 3.. The mean weight gain and specific growth rate reduced with increase in concentration. The highest weight gain was recorded in the control group of both fish and rats compared with the exposed groups of fish and rats.

Significant increase in the transaminases (ALT and AST) activities observed in the blood of fish and rats exposed to WSFD could be due to possible leakage of enzymes across damaged liver cells and increase synthesis of enzymes by the liver (Abdel Razik and Shehata, 2007). The liver being the center for detoxification of foreign compound that enters the body made it to be exposed to environmental toxins and chemicals present in food or drink. Similar reports were given by Oluah, 1998, 1999, Rao, 2006 and Zikic, *et al*., 2001 and also shalby, 2006.

In this study, the decrease in the values of ALT and AST in the gill (WSFD) connotes possible tissue damage. This is also supported by Soufy et al, 2007 who carried out investigation on the biochemical and pathological effect on monosex tilapia *Oreochromis niloticus* on chronic exposure to carbofuran. Increased Values of LDH in the gill and decreased values in the liver of rats are similar to the findings of Venkateswara, (2008). Oluah, *et al* .(2005) recorded increased in activity of serum and liver dehydrogenase (LDH) enzyme in C. *albopunctactus* exposed to

increasing concentrations of sub lethal Gammalin 20 and Acetellic 25 EC. This has supported the present result of increased LDH in the liver of the fish. The increased LDH activity in fish exposed to WSFD observed in this study may reflect the increased rate of conversion of lactate to pyruvate and then to glucose. Saha, *et al*. (1999) reported that a stressed fish *(O. massambicus*) exposed to phenol may continuously move opercula when in need of oxygen, which may induced the anaerobic oxidation to release energy by enhanced LDH activity.

Activities of creatinine increased as the concentration increased in both fish and rats this is in agreement with Nwamba, *et al*. (2006) and Ugwu, *et al*.(2007) who reported increased in activities of amylase and creatinine kinase enzymes in *Heterobranchus biodorsalis* juveniles as concentration of Bonny-light crude oil increased from 1.00 to 8.00ml$^{-1.}$ In this study, hepatic SOD activity was induced significantly during almost all 28 days in fish and 30 days in rats. Induction of SOD could occur during high production of superoxide onion radical. Increase in the SOD activity leads to an increase in oxygen production. SOD was said to be very sensitive to oil pollution. Zhang, *et al*., (2003) reported that SOD act as an early monitor in an oil-dominant aquatic ecosystem. The result of this present study has also indicated that SOD activity is very sensitive to etroleum pollutions. This is an indication that petroleum hydrocarbon (WSFD) are potent mediators of free radical formation in fish and that increase in catalase activity in all the tissues examined can represent an adaptive response to protect the fish from the toxicity of free radicals induced by these hydrocarbons. This is in agreement with the works of Achuba and Osakwe, (2003); Zang, *et al*. (2003); Zang, *et al.* (2004); Simonato, *et al.*,(2006).

Studies have revealed that when water quality is affected by toxicant, physiological changes will be observed in the values of one or more of the haematological parameters (Van Vuren, 1986). The present study has demonstrated that long term exposure of *Heteroclarias* and rats to Diesel oil induced anaemia, and as well revealed that Heteroclarias exposed to sub-lethal concentrations of WSFD clearly recorded a dose-dependent reduction in RBC, HB, PCV and MCV as concentration of the toxicant increased, in which all were significantly lower ($p<0.05$) than the control group. This could be due to haemolysis, which is caused by excessive destruction of RBC's which led to decrease HB content. The trend obtained in this study is in accordance with the reports of Mohssen (1997); Dede and Kagbo (2001); Bhatia, *et al*. (2004); Kori-Siakpere, *et al.* (2005); then Patnaik and Patra, (2006). MCH value showed an increased trend on prolonged exposure of fish to WSFD. Similar reports were given by some earlier workers, Gill, *et al.* (1991); Duta *et al.* (1992); Bhataia, *et al*. (2004). This anaemic response was also said to be as a result of destruction or the inhibition of erythrocyte production, haemodilution and could be as a result of the destruction of intestinal cells by the toxicant (Samprath, *et al*., 1993; Saheny and Johal,(2000); Patriak and patra, (2006). However, the decrease in the erythropoietic activity of the kidney (Santhakiamr, et al., 1999) or the haemodilution resulting from impaired Osmoregulation across the epithelium (Wedemeye et al., 1984) was said to be because of decreased in RBC level of *Clarias gariepinus* exposed to Paraquat concentrations.

Table 1: Enzymes activities in blood, gill, and liver of *Heteroclarias* exposed different concentration each of Monocrotophos (MCP) for 28 days.

		Blood					Gill				Liver			
Toxicant	Conc.	ALT Unit/g	AST Unit/g	CERAT. Unit/g	LDH Unit/g	SOD Unit/g	ALT Unit/g	AST Unit/g	LDH Unit/g	SOD Unit/g	ALT Unit/g	AST Unit/g	LDH Unit/g	SOD Unit/g
MCP	(NGL)													
	0.00	0.41+0.00^{c}	0.39+0.00^{b}	0.48+0.01^{c}	0.30+0.00^{b}	1.65+0.00^{a}	0.65+0.01^{a}	0.72+0.01^{d}	0.55+0.01^{a}	0.01+0.06^{b}	25.75+0.03^{a}	17.70+0.06^{a}	2.25+0.03^{bc}	0.02+0.06^{a}
	0.15	0.21+0.01^{f}	0.31+0.01^{a}	0.50+0.01^{b}	024+*0.01*^{d}	1.80+0.011^{cd}	1.25+0.01^{d}	1.02+0.01^{c}	0.75+0.01^{bc}	0.01+0.00^{b}	17.50+0.01^{d}	23.02+0.01^{a}	2.35+0.03^{b}	0.02+0.06^{a}
	0.20	0.37+0.01^{d}	0.51+0.01^{a}	0.50+0.01^{b}	0.22+0.00^{d}	2.10+0.01^{bc}	1.20+0.01^{d}	1.10+0.00^{c}	0.80+0.06^{ab}	0.01+0.00^{b}	25.50+0.01^{a}	21.05+0.06^{c}	2.25+0.01^{bc}	0.02+0.06^{a}
	0.25	0.48±0.01^{a}	0.31±0.01^{a}	0.39±0.01^{a}	0.27±0.01^{c}	1.95±0.01^{cd}	1.45±0.03^{c}	1.50±0.03^{b}	0.90±0.06^{a}	0.02±0.06^{a}	19.15±0.03^{c}	22.62±0.01^{b}	2.16±0.01^{c}	0.02±0.06^{a}
	0.30	0.46+0.01^{b}	0.34+0.01^{d}	0.42+0.01^{d}	0.38+0.01^{a}	2.50+0.20^{a}	1.60+0.06^{b}	1.85+0.03^{a}	0.65+0.03^{cd}	0.02+0.06^{a}	21.05+0.03^{b}	20.50+0.12^{d}	2.45+0.03^{a}	0.02+0.06^{a}
	0.35	0.24+0.01^{e}	0.36+0.01^{c}	0.56+0.01^{a}	0.32+0.01^{b}	2.30+0.01^{a}	1.75+0.03^{a}	1.85+0.01^{a}	0.65+0.01^{cd}	0.02+0.06^{a}	10.35+0.33^{e}	12.60+0.06^{f}	1.70+0.06^{d}	0.01+0.06^{b}

Table 2 :- Enzymes activities in blood, kidney and liver of Rat fed with diet containing fish exposed to different concentrations of Monochrotophos (MCP) for 30 days.

		Blood					Kidney				Liver			
Toxicant	Conc.	ALT unit/g	AST unit/g	LDH unit/g	CREAT unit/g	SOD unit/g	ALT unit/g	AST unit/g	LDH unit/g	SOD unit/g	ALT unit/g	AST unit/g	LDH unit/g	SOD unit/g
MCP	(µ/g)													
—	0.00	0.27±0.01^{g}	0.23±0.01^{f}	2.10±0.34^{g}	0.56±0.01^{f}	3.15±0.01^{a}	6.50±0.06^{f}	7.05±0.03^{g}	12.05±0.03^{c}	0.03±0.01^{a}	9.40±0.06^{f}	12.05±0.03^{f}	18.95±0.03^{f}	0.04±0.06
	0.15	0.36±0.01^{d}	0.31±0.01^{g}	2.60±0.01^{d}	0.61±0.01^{g}	2.69±0.02^{b}	9.50±0.06^{d}	6.85±0.06^{f}	12.20±0.06^{c}	0.03±0.06^{a}	10.25±0.03^{g}	12.80 ±0.06^{g}	22.10±0.06^{d}	0.03±0.01
—	0.20	0.41±0.01^{c}	0.36±0.01^{d}	2.45±0.01^{d}	0.72±0.01^{c}	2.47±0.01^{d}	8.74±0.02^{g}	8.22±0.01^{d}	12.32±0.01^{b}	0.02±0.03^{b}	12.58+0.02^{d}	13.65±0.03^{d}	22.89±0.02^{c}	0.04±0.01
	0.25	0.41±0.01^{c}	0.39±0.01^{c}	2.65±0.01^{d}	0.82±0.01^{a}	2.51±0.01^{c}	10.35±0.03^{b}	9.40±0.06^{b}	12.70±0.12^{b}	0.02±0.01^{b}	13.04±0.02^{d}	13.37 ±0.01^{b}	21.90±0.00^{g}	0.04±0.01
	0.30	0.52±0.01^{a}	0.50±0.01^{b}	3.10±0.01^{b}	0.66±0.01^{d}	2.05±0.01^{g}	9.70±0.06^{c}	9.32±0.02^{c}	12.40±0.06^{bc}	0.02±0.01^{b}	15.50±0.28^{b}	14.40±0.12^{b}	23.18±0.02^{b}	0.04±0.01
	0.35	0.47±0.01^{b}	0.52±0.01^{a}	3.25±0.01^{b}	0.75±0.01^{a}	1.90±0.01^{f}	12.35±0.03^{a}	9.80±0.06^{a}	26.50±0.29^{a}	0.02±0.01^{b}	16.89±0.06^{a}	14.97±0.01^{a}	23.75±0.03^{a}	0.05±0.01

Values are means off two replicates ± SEM (standard error mean). Column Values with different superscripts are significantly different($P < 0.05$).

Table 3:- Haematological Parameters of *Heteroclarias* exposed to six different concentrations each of Monochrotophos (MCP) for 28 Days

Toxicant	Conc (µg/l)	Rbc x 10^{12}/l	Hb (g/dl)	Pcv (%)	MCV(µl)	MCH(pg)	MCHC(g/dl)	WBCx10^{9}/l	Total protein(g/l)	Glucose (Mmol/l)
MCP										
	0.00	3.80±5.77^{a}	9.10±5.77^{a}	28.00±0.58^{b}	82.00±1.15^{a}	28.00±0.58^{b}	33.00±0.58^{a}	1.90±5.77^{d}	86.00±0.58^{c}	6.60±5.77^{b}
	0.15	3.69±5.77^{b}	9.00±0.00^{b}	26.00±0.58^{bc}	80.00±1.15^{ab}	29.00±1.15^{ab}	30.00±0.58^{b}	2.40±5.77^{b}	110.00±0.58^{c}	5.20±0.12^{d}
	0.20	3.66±1.16^{c}	9.00±5.77^{a}	27.00±58^{ab}	78.00±1.15^{b}	30.00±1.15^{ab}	29.00±0.58^{b}	2.80±5.77^{d}	78.00±0.58^{d}	8.50±5.77^{a}
	0.25	3.65±5.77^{c}	7.70±5.77^{a}	27.00±58^{ab}	81.00±0.58^{ab}	31.00±0.58^{ab}	32.00±0.58^{a}	2.00±5.77^{cd}	89.00±0.58^{bc}	6.40±5.77^{bc}
	0.30	3.62±1.16^{d}	7.40±5.77^{c}	25.00±0.58^{cd}	79.00±0.58^{ab}	30.00±0.58^{ab}	30.00±0.58^{b}	2.10±0.00c	96.00±0.58^{b}	6.20±0.00^{c}
	0.35	3.55±5.77^{e}	7.00±0.00^{d}	24.00±0.58^{d}	81.00±0.58^{ab}	32.00±1.15^{a}	32.00±0.58^{a}	1.80±0.12^{d}	87.00±0.58^{c}	5.3±5.77^{d}

Table 4:- Haematological parameters of *Heteroclarias* exposed to six different concentrations each of Monochrotophos (MCP) for 30 days.

Toxicant	Conc	Rbc x 10^{12}/1	Hb (g/d1)	Pcv (%)	MCV(µl)	MCH(pg)	MCHC(g/d1)	WBC x 10^{9}/1	Glucose (Mmo1/l)
MCP	(ug/l)								
	0.00	5.19±5.77^{a}	16.30±5.77^{a}	50.00±0.58^{a}	94.00±0.58^{b}	31.00±0.58^{a}	33.00±0.00^{a}	4.00±0.00^{t}	3.50±5.77^{a}
	0.15	5.12±8.82^{b}	16.00±0.00^{b}	49.00±0.58^{ab}	96.00±0.58^{a}	32.00±0.58^{a}	33.00±0.00^{a}	6.20±5.77^{c}	4.20±0.12^{d}
	0.20	5.02±1.16^{c}	15.3±5.77^{c}	48.00±0.58^{b}	94.00±0.58^{b}	31.00±0.58^{a}	33.00±0.00^{a}	12.80±5.77^{a}	3.90±5.77^{e}
	0.25	4.84±1.16^{d}	15.30±5.77^{c}	46.00±0.58^{c}	96.00±0.58^{a}	32.00±0.58^{a}	33.00±0.00^{a}	12.00±0.00^{b}	4.60±5.77^{c}
	0.30	4.27±1.16^{e}	13.30±5.77^{d}	4.00±0.58^{d}	96.00±0.58^{a}	32.00±0.58^{a}	33.00±0.00^{a}	6.00±0.00^{d}	4.80±5.77^{b}
	0.35	2.24±5.77^{t}	5.70±5.77^{e}	17.00±0.58^{e}	17.00±0.58^{c}	26.00±0.58^{c}	33.00±0.00^{a}	4.90±5.77^{e}	5.20±5.77^{a}

Values are means of two replicates ± SEM (standard error mean).Column values with different superscripts are significantly different ($P < 0.05$).

Table 5: Mean weight of *Heteroclarias* exposed to six different concentrations each of Monochrotophos (MCP) for 28 days.

		Concentration (µg/l)					
Toxicant	Parameters (g)	Control	0.15	0.20	0.25	0.30	0.35
MCP	Average Initial weight	580±23.09[a]	553±37.11[b]	603±31.80[c]	593±21.86[d]	576±52.39[e]	573±7.64[f]
	Average final weight	1340±10.00[a]	1296±26.03[b]	1280±30.00[c]	1266±16.67[d]	1250±28.87[e]	1070±15.28[f]
	Weight gain	760±2.89[a]	743±8.82[b]	677±8.82[c]	673±21.86[d]	674±5.30[e]	497±2.89[87]
	% weight gain	131	134	112	113	117	1.004
	Specific growth rate btw day 1 and day 28	1.347	1.370	1.211	1.22	1.246	

.

Values are means of two replicates ± SEM (standard error mean). Column values with different superscripts are significantly different ($p < 0.05$).

Table 6: - Average weight of rat exposed to six different concentrations each of Monochrotophos (MCP) for 30 days.

		Concentration (µg/l)					
Toxicant	Parameters (g)	Control	0.15	0.20	0.25	0.30	0.35
MCP	Average Initial weight	800±28.87[a]	1000±11.55[b]	900±28.87[c]	875±14.43[d]	750±28.87[e]	925±2.89[f]
	Average final weight	1680±17.32[a]	1400±0.00[b]	1200±57.73[c]	1500±86.06[d]	1300±0.00[e]	1625±0.00[f]
	Weight gain	880±23.09[a]	400±0.00[b]	300±0.00[c]	625±2.89[d]	550±5.77[e]	700±0.00[f]
	% weight gain	110	40	33	71	73	76
	Specific growth rate btw day 1 and day 30	1.111	0.504	0.431	0.807	0.823	0.844

Values are mean of two replicates ± SEM (standard error mean). Column values with different superscripts are significantly different ($p < 0.05$).

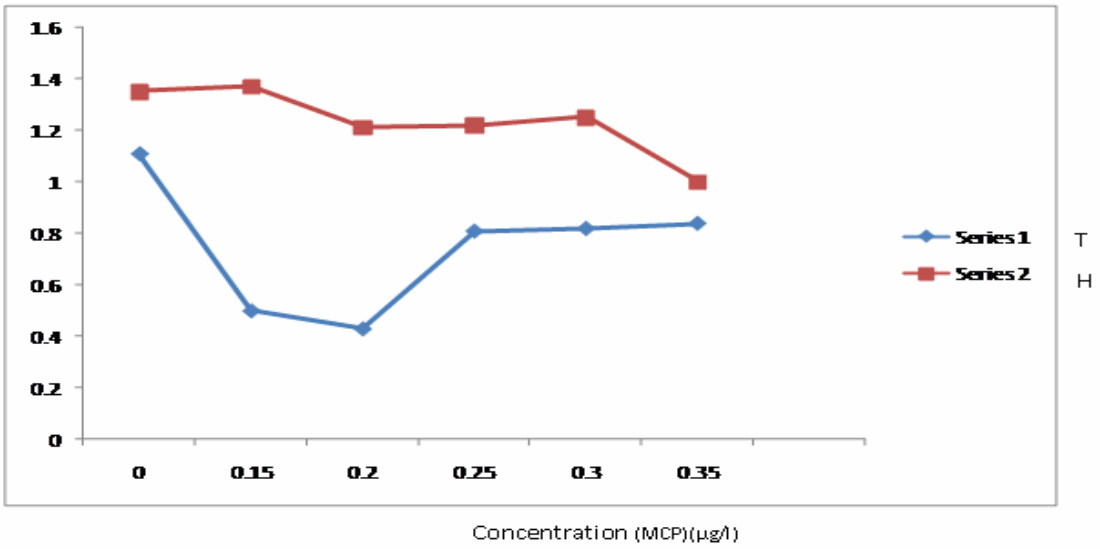

Fig.13 Specific growth rate of *Heteroclarias* exposed to varying concentration of MCP for 28 days and *Albino Rat fed on fish contaminated with MCP for 30* days

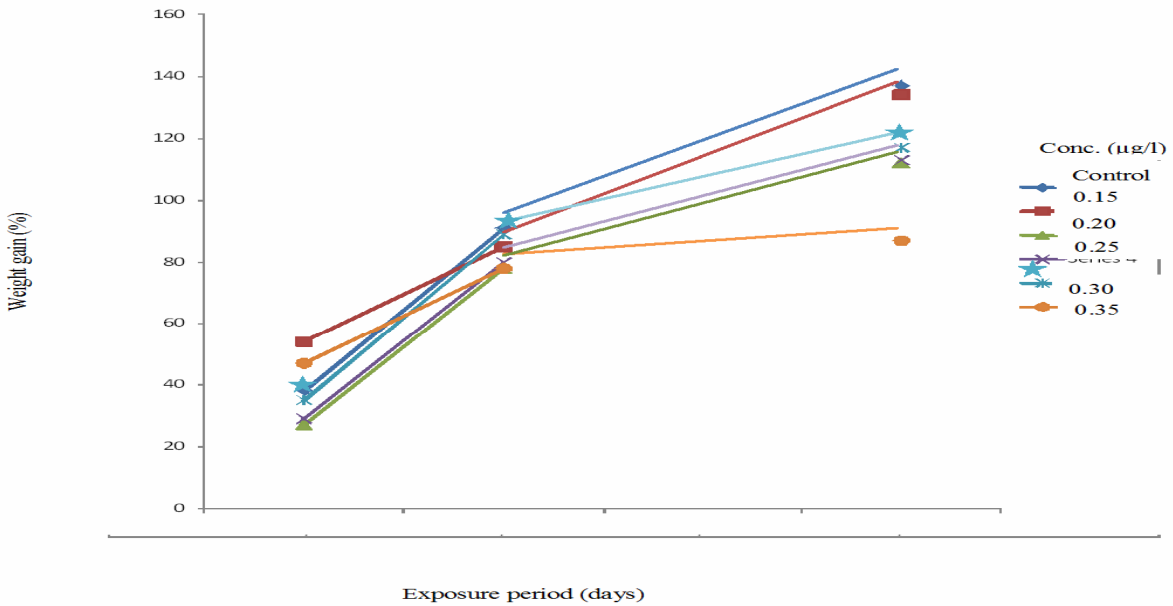

Fig. 15 Mean weight gain of *Heteroclarias* exposed to varying concentrations of MCP for 28 days

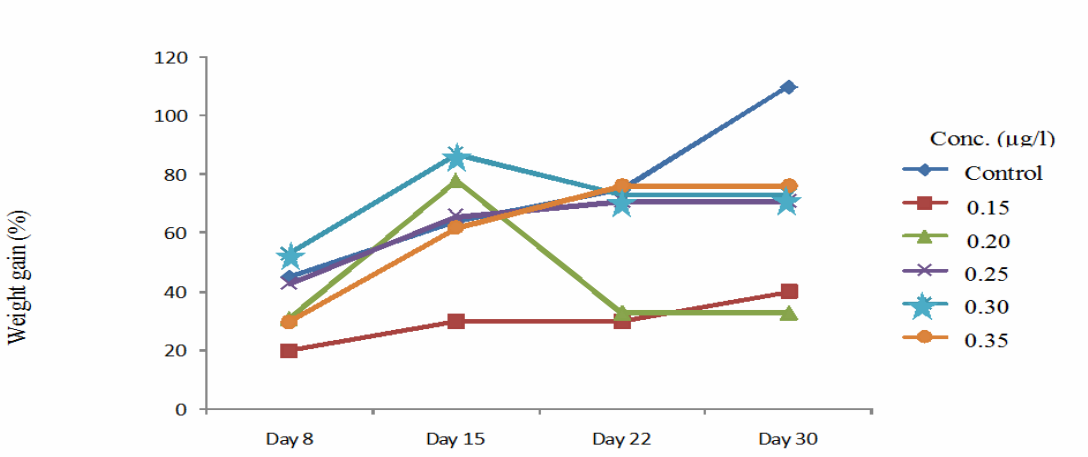

Fig. 17 Mean weight gain of *Albino Rats fed on fish contaminated with MCP for 30* days

The increased white blood cell count can be correlated with an increase in antibody production which helps in survival and recovery of fish exposed to sub-lethal concentration of pesticides (Joshi *et al*., 2003). Seth and Saxena, (2003) reported an increased in total leucocytes count in *Channa punictatus* exposed to Fenvalen intoxication. The significant rise in WBC may be considered as defensive mechanism triggered by the immune system in the fish and rats. WBC plays an important role in the immune system of living organisms. An increase in total leucocyte counts (TLC) thus occurs as a protective response to stress induced by the toxicants (Das 1998).

The reduction of glucose content in the blood may be that most of the blood glucose must have been used-up because of the greater energy demand by the fish due to stress induced by MCP intoxication, the increased glucose content in the blood of rat may be because of the energy demand induced by the toxicant WSFD.

These responses can be considered adaptive processes that help the organism with increased energy demand during exposure to stress factors (Martinez and Colus, 2002). Protein metabolism can provide information on the general energy mobilization of an animal and show relationships with effects of contaminant in these organisms (Adams, *et al*., 1990).This may be that more protein was metabolized and released into the blood stream to be used as an alternative source of energy demand due to stress induced by MCP intoxication and this agrees with observation of Juliana *et al.* (2008).

Reduction in specific growth rate with increased WSFD concentrations could be due to suppressive effect on the food consumption (rats) by the toxicant or increased activity in an attempt to avoid polluted water (fish). These results were in high standard with the reports of (Logasulamy, 1997) and (Avaoja and Oti, 1997). Also the growth reduction observed in this study could have been due to a

reduced oxygen carrying capacity and stimulation of erythropoiesis of the blood as a result of reduction in cellular haemoglobin which in turns result in inefficient utilization of assimilated food or inhibition of certain enzymes of the metabolic pathway as was reported by (Hodson *et al.*, 1978).

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