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Nandagopalan Geetha
Department of Zoology (PG), PSG College of Arts and Science, Civil Aerodrome (Post), Coimbatore, Tamil Nadu, India

Naveena Priya
Department of Zoology (PG), PSG College of Arts and Science, Civil Aerodrome (Post), Coimbatore, Tamil Nadu, India

Correspondence
Nandagopalan Geetha
Department of Zoology (PG), PSG College of Arts and Science, Civil Aerodrome (Post), Coimbatore, Tamil Nadu, India

Impact of triclosan an antimicrobial agent on haematological parameters of fresh water fish Labeo rohita

Nandagopalan Geetha and Naveena Priya

Abstract
Pharmaceuticals and personal care products are emerging as class III environmental pollutants. Among such pharmaceutical chemicals, Triclosan (TCS) is the most extensively used antibacterial agents, which inhibits the activity of bacteria, virus and fungi. From industrial effluents TCS reach aquatic ecosystem and accumulated in non- target aquatic organisms especially fish. Then, ultimately reach human beings through food chain. In the present study, toxic effect of triclosan on fresh water fish Labeo rohita was evaluated by estimating haematological parameters. Static bio- assay test was performed to determine the median lethal concentration (LC_{50}) of TCS for 24hours, which was 0.9mg/L. Fish were exposed to sub-lethal concentration(0.09mg/L) for 15 days. At stipulated time (5^{th}, 10^{th} & 15^{th} day) haematological parameters were estimated. When compare to control group, there was a significant($p < 0.05$) decrease in Haemoglobin (Hb) content, Erythrocyte count (RBC), Haematocrit (HCT) and Mean Corpuscular Volume (MCV) in TCS exposed fish, which indicates the disruptive action of TCS on erythropoitic tissues, causing shrinkage in RBC cells and making the erythrocytes more brittle and porous. Whereas significant (($p < 0.05$) increase in Leucocytes count (WBC), Mean Cell Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) indicates the direct or indirect responses of structural damage in RBC membranes resulting in haemolysis. Bi-phasic response in WBC count may be due to stimulation in immune system in response to TCS, or hypoxia and gill damage due to accumulation of toxicant in gills, whereas at 15 th day WBC count was declined and the possible reason for such decline may be due to leucocytosis or non-specific decline in immunity (leucopenia) which are the characteristic response in animals exhibiting stress.

Keywords: Triclosan, sub lethal toxicity, haematology, labeo rohita

Introduction
Triclosan (TCS) 5-chloro – 2 – (2, 4 dichlorophenoxy) phenol is a synthetic broad spectrum antibacterial or antimicrobial agent which inhibits the activity of bacteria, virus and fungi. Hence triclosan is widely used as one of the ingredients in numerous personal care products such as tooth pastes, antibacterial soaps, cosmetics and antiseptic products. From cosmetic industrial effluents and discarded personal care products TCS ultimately reach aquatic ecosystem and enters the non-target organism through food chain [1]. A major environmental concern is the tendency of TCS to be transformed into a series of chlorinated triclosan derivatives (CTDs) [2]. TCS causes a drastic effect in humans. Geens ***et al***. [3] investigated the absorption and bioaccumulation in humans when exposed to Triclosan and tetrabromobisphenol. TCS, is an endocrine disruptor [4,5], affects mitochondrial function [6], disrupt calcium signalling [7] and zinc homeostasis [8], alters immunological parameters [9] and affects embryonic development [10]. An allergic reaction test was observed in sensitised patients during the exposure of triclosan [11].
In addition, TCS may be bio-transformed into methyl-triclosan, a more persistent compound than the parent compound, by biological methylation [12]. Ability of certain seafood to accumulate TCS is a route of exposure to humans [13]. TCS has been studied using several types of environmentally sensitive species including microalgae and fungi. Few studies revealed that TCS exhibited terotogenic responses; hatching delay and mortality in embryos and larva of zebra fish, but not much works have been reported on fish haematological and biochemical parameters. In the environment triclosan is more toxic because of its structure, which resembles to the endocrine hormones structure and cause carcinogenic effect in non-

target organisms including human [14, 15]. High TCS level was recorded in largest Indian rivers like Kaveri, Tamiraparani and Vellar [16], where economically important fishes including major carps are nurtured and riverine capture fisheries are very common practice. Furthermore, information on TCS accumulation and toxicity to freshwater fishes is very scanty. Hence the present study was designed to find out the median lethal concentration of Triclosan (LC50) for 24 hrs and its sub lethal toxic impact on haematological parameters of fresh water fish *Labeo rohita*, because haematological parameters give early warning of the health status of the animal.

Material and methods

Chemicals

Analytical grade TCS, 5-Chloro – 2 (2,4 dichlorophenoxy) phenol (97%) purity and dimethyl sulfoxide (DMSO,99.99%) purity were purchased from Hi- media research Laboratory, INDIA. TCS was dissolved in DMSO to make a stock solution at a concentration of 1000mg/L^{-1}. Positive control of DMSO was kept below 0.1%.

Acclimatization and maintenance of fish

Healthy and active fingerlings of Labeo rohita were procured from Tamil Nadu Fisheries Development Corporation Fish Farm, Aliyar, Tamil Nadu, India. Fish fingerlings of 7- 8 cm length and 5±0.80 g weight were transported in eco-friendly disposable bags with oxygenated water. Later, they were acclimatized for 10days at 25± 2^0C in large cement tank of 1500 L capacity disinfected with potassium permanganate and washed thoroughly prior to the introduction to fish to prevent fungal infection. Since physico-chemical parameters have significant influence on biodegradability and toxicity of pollutants, they were estimated and maintained throughout the experiment (Dissolved Oxygen 4.27 ± 0.08mg/L; Total alkalinity 130 ± 0.9mg/L; Salinity 0.13 ± 0.054 ppt; Total hardness 40 ± 0.6 mg/L; calcium 30 ± 0.95 mg/L; Magnesium 10 ±0.8 mg/L). During acclimatization, fish were fed ad libitum with rice bran and ground nut oil cake in 2: 1 ratio. Feeding was given at least 1 hour prior to the replacement of water and removed the excess feed and faecal matters in the tank. This ensures sufficient oxygen supply for fish and the environment is devoid of any accumulated metabolic waste. Feeding was stopped 24hrs prior to the experiment

Median lethal concentration and behavioural study

Preliminary toxicity tests were conducted (data not shown) to find the Median Lethal Concentration of TCS for 24 h. 10 fish were randomly selected from the stock for each experiments. After 24 h, mortality rate was recorded and 50% mortality was observed in 0.9mg/L. Narrow range concentration was repeated to confirm the 24 h static renewal acute toxicity definitive test [17]. Behavioural changes were noted throughout the experiments. For sub-lethal toxicity study, 3 glass Aquaria (100 L capacity) filled with chlorine free tank water were used. Fish were randomly selected from the stock and housed 70 fish in each tank; 0.09mg/L (Sublethal treatment) of TCS was introduced into the aquarium by removing equal volume of water. At the end of every 24 h, water was renewed and freshly prepared TCS solution was added to maintain the TCS at a constant level. Similarly, after stipulated period, the TCS dose was adjusted corresponding with remaining number of fish to maintain the same TCS concentration (0.09mg/L) throughout the study period. Remaining two tanks were used for control (Without Toxicant) and Positive/ Solvent control (DMSO) for 15 days. Three replicates were also maintained simultaneously with treatment and control groups with same set up.

Fish sampling and haematological analysis

At the end of stipulated time, blood sample were collected from experiment and control groups using plastic disposable syringe fitted with 26 gauge needle which was already moisture with heparin ((Beparine®, heparin sodium, IP 5000 IU $ml^{-}1$, derived from beef intestinal mucosa containing 0.15% W/V cholesterol IP preservative) an anticoagulant. Blood samples (20 fish) from experiment and control groups were stored in separate plastic vials moisture with heparin for haematological analysis. Such as erythrocyte (RBC) and leukocyte (WBC) counts [18], hemoglobin (Hb) [19], and hematocrit (HCT) [20]. The other hematological indices like mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated using standard formulas.

Statistical Analysis

Data are expressed as Mean ± SE and were analysed using statistical software SPSS version 20. Significance of sample means was evaluated by using Two- way ANOVA following Duncan's Multiple Range Test (DMRT) to determine the level of significance at $p < 0.05$.

Results and discussion

Triclosan is highly toxic to *Labeo rohita*. During the acute toxic study fish displayed altered behavioural changes such as, circular movements, erratic and fast swimming around the trough, backward movement, jerking movement around the trough and floating upside down before they died. Similar observations have been reported by Fritsch et al. [21]. The observed abnormal swimming pattern may be due to impairment in caudal fin and neurological dysfunction caused by Triclosan toxicity [22]. Even though mortality was not occurred during sub-lethal study (0.09mg/L), significant decrease in the number of RBC cells, Hb, HCT, MCV and increase in WBC count, MCH and MCHC were recorded.

Decrease in the number of RBC cells [Fig.1] during sub-lethal treatment indicates that TCS induced shrinkage in RBC cells and making the erythrocytes more brittle and porous. Similar report in *Oreochromis niloticus* exposed to triclosan reported by Vijitha et al [23], may find good support for the present study. There is no significant change was observed throughout the study period for all the parameters between control and positive control groups. This clearly indicates that DMSO is not toxic to fish. Whereas, significant difference ($p < 0.05$) between the control groups and triclosan treated fish were noted. Significant increase in WBC count may be due to stimulation in immune system in response to TCS, or hypoxia and gill damage due to accumulation of toxicant in gills, whereas at 15^{th} day WBC count [Fig.2] was declined when compare to 10^{th} day of treatment and the possible reason for such decline may be due to leucocytosis or non-specific decline in immunity (leucopenia) which are the characteristic response in animals exhibiting stress [24].

Decrease in Hb content [Fig.3], RBC count and HCT (%) [Fig.4] in the present study indicates severe anaemia. Similar findings were reported in *Oreochromis niloticus* [23] exposed to triclosan. Such decline may be due to the disruptive action of triclosan on erythropoitic tissues such as kidney and spleen which in turn cause decrease in RBC count or swelling of RBC as well as poor mobilization of Hb from the spleen and other haematopoietic organs.

RBC indices include MCV, MCH and MCHC. MCV refers to the average size of the RBC constituting the sample. MCH refers to the weight of the Hb in the average RBC of the sample. MCHC refers to the average concentration of Hb in a given volume of packed red cells (HCT %) of the sample. Decrease in MCV [Fig.5] throughout the exposure period and the severe decline at the end of 5th day treatment of the present study may be due to the shrinkage of RBC or production of large number of lymphocytes. Direct or indirect responses of structural damage to RBC membranes might have caused haemolysis that could contribute for high MCH [Fig.6] and MCHC in the present investigation. Likewise, within the experimental group, moderate decrease in MCHC [Fig.7] at the end of 10th day may be due to great loss of Hb. Similar findings have been reported by Barot and Bahadur [25] and Srivastav and Roy [26]. Even though, mortality was not recorded throughout the sub-lethal Triclosan treatment, alteration in haematological parameters indicates that Triclosan is toxic to fish and can able to affect the immunity in fish.

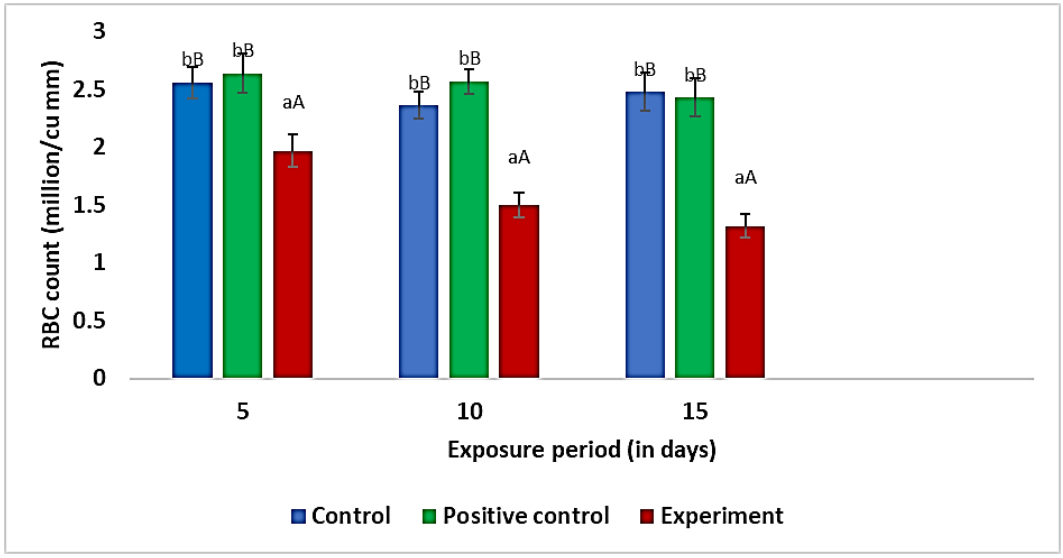

Fig 1: Bar diagram showing changes in the RBC count of fresh water fish Labeo rohita exposed to sublethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

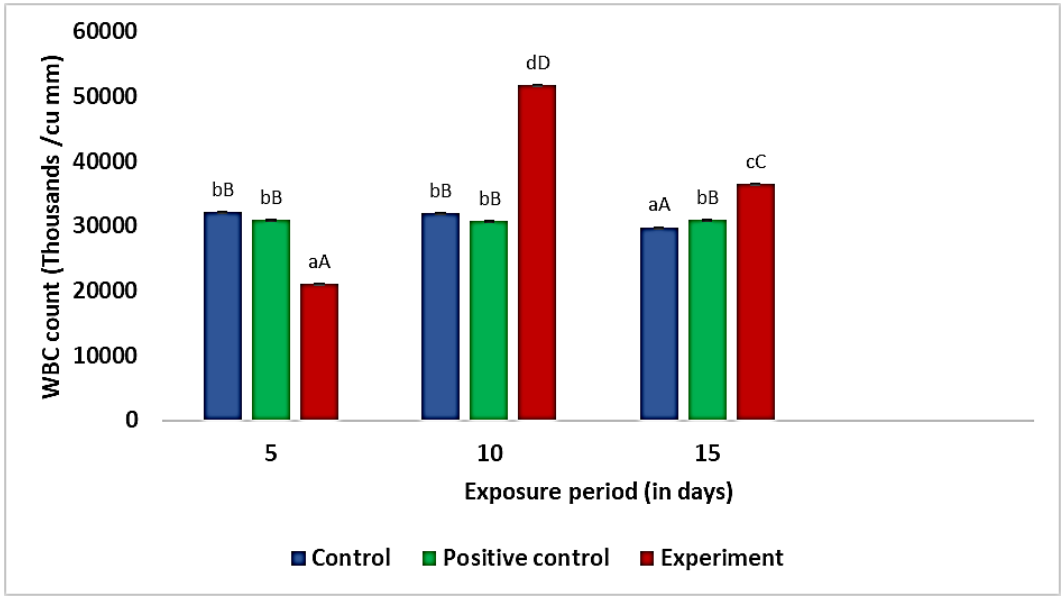

Fig 2: Bar diagram showing changes in the WBC count of fresh water fish Labeo rohita exposed to sub -lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

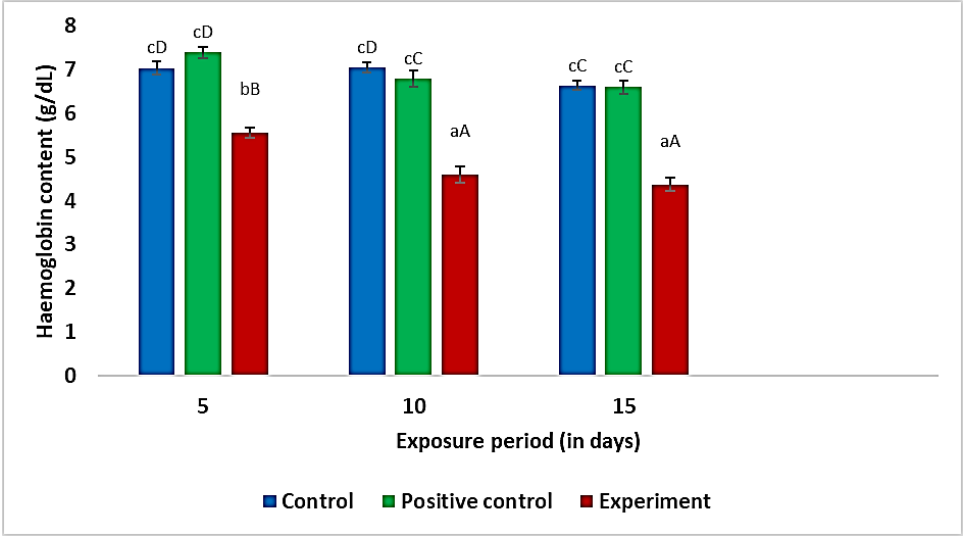

Fig 3: Bar diagram showing changes in the Hb content of fresh water fish Labeo rohita exposed to sub lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

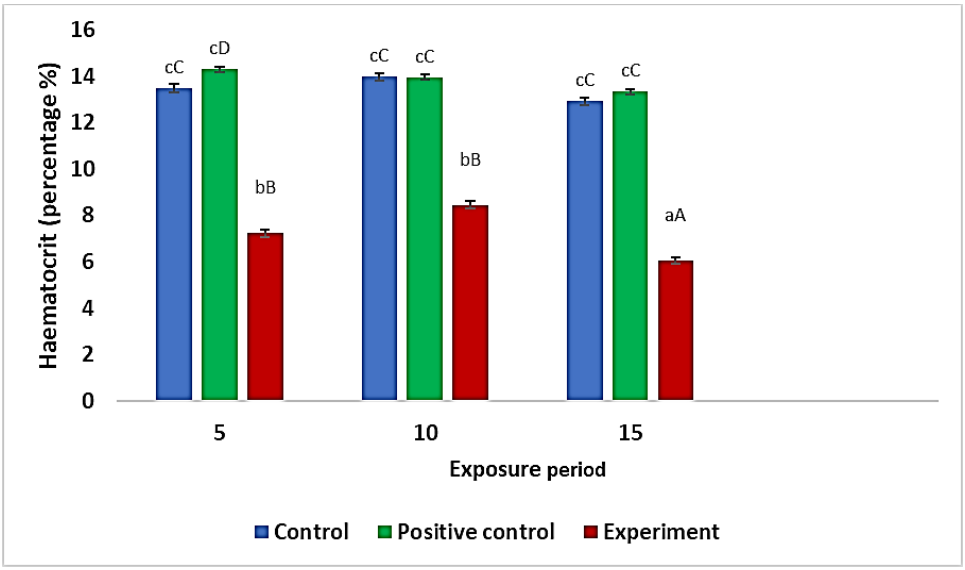

Fig 4: Bar diagram showing changes in the HCT % of fresh water fish Labeo rohita exposed to sub lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

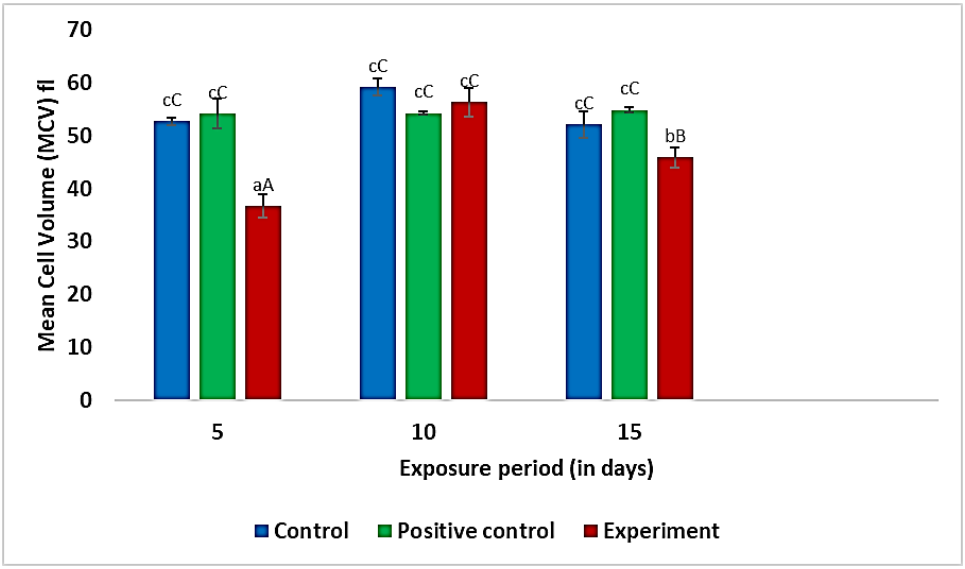

Fig 5: Bar diagram showing changes in the MCV (in fl) of fresh water fish Labeo rohita exposed to sub lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

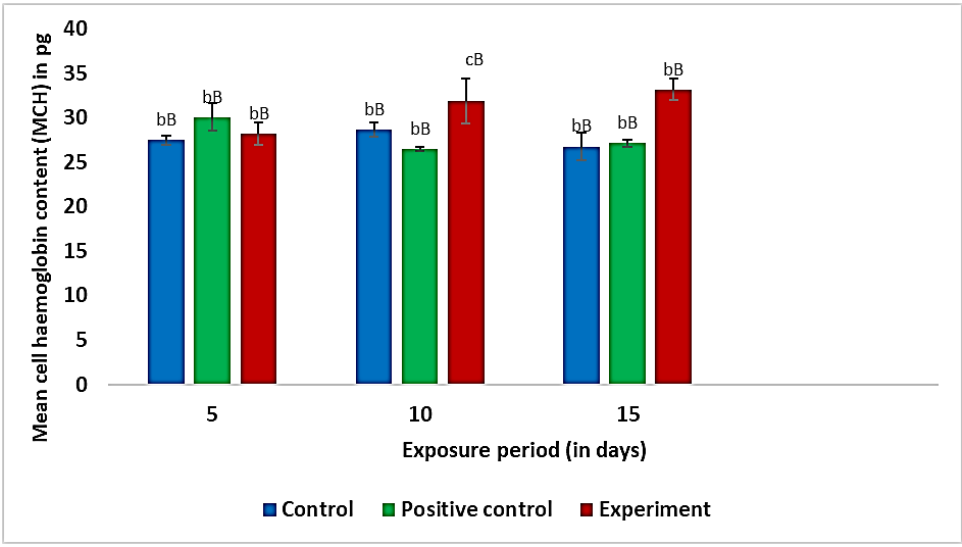

Fig 6: Bar diagram showing changes in the MCH (in pg) of fresh water fish Labeo rohita exposed to sub lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

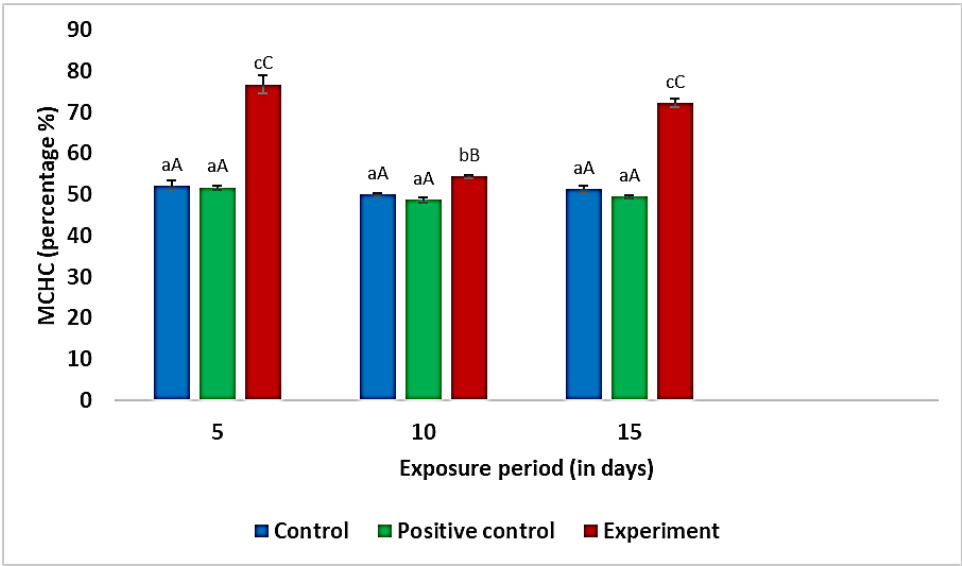

Fig 7: Bar diagram showing changes in the MCHC % of fresh water fish Labeo rohita exposed to sub lethal concentration of triclosan (0.09mg/L) for 15 days. Values with different letters (lower case) differ significantly ($p < 0.05$) between different periods for a particular treatment. Values with different letters (upper case) differ significantly ($p < 0.05$) between different treatments for a particular period.

Conclusion

Based on the present study, Triclosan is highly toxic and triggered the secondary stress responses in Labeo rohita. The severity of changes in the haematological parameter is directly proportional to the exposure period of fingerlings to the Triclosan. According to Food and Drug Administration up to 0.3% Triclosan is permitted in tooth paste and in liquid hand soaps its concentration ranges from 0.1 – 0.3%. But the present study emphasis that, 0.09 mg/L itself is affecting the health status of the fingerlings. Analysis of haematological parameters gives the health status of organism and a useful biomarker for eco- toxicological risk assessment. Furthermore, if fish from such polluted water gone for human consumption, definitely affect human health and can be a potential carcinogen to human beings.

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