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Toxicological effect of mercuric chloride on *Heteropneustes fossilis* (Bloch) with reference to behavioural and haematological alteration

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Abstract

The present investigation deals with the toxicological effect of mercuric chloride on a model fish organism *Heteropneustes fossilis* on haematological parameters as well as behavioural changes keeping most of the physicochemical parameters in the experimental aquariums are at optimal level. The LC_{50} concentration where 50% death of the fishes occur is experimentally carried out which is found to be 0.10 mg/l, a very lesser quantity, thus showing the level of toxicity of mercuric chloride. The fishes were treated at sub lethal concentration of LC_{50} ($1/5^{th}$ of LC_{50} concentration i.e. 0.02 mg/l) for a period of 24hrs, 48hrs, 72hrs and 96hrs to get haematological alteration. The result shows that RBC and Haemoglobin decline significantly as the increase of time of exposure of mercuric chloride. Haemoglobin % was observed to drop about 50% after 96 hours of exposure of mercuric chloride. However, WBC is found to be increased with time initially and attain maximum at 48 hours of exposure and steadily decline thereafter. Differential Leucocytes Count (DLC) shows an increasing trend as the exposure of mercuric chloride increases. Lymphocytes shows an increasing trend from lower to higher as exposure time increases with more or less similar trend with Basophil, where the highest peak is observed at 48 hours exposure time and then decline slowly. On the other hand Eosinophil, Neutrophil and Monocytes show a reverse phenomenon where the number of counts decreases steadily as exposure time increases. The various behavioral changes like restlessness, abnormal swimming behavior, vigorous jerks of body, loss of balance, surfacing, encircling, mucus secretion and anorexia observed in *Heteropneustes fossilis* indicates the effect of the level of toxicity of mercuric chloride.

Keywords: Mercuric chloride, *Heteropneustes fossilis,* haematological parameters, LC_{50}, Sub lethal concentration

1. Introduction

Now a day's pollution is considered to be a serious threat globally due to urbanization and industrialization. Aquatic environment is too constantly polluted from variety factors including natural process as well as anthropogenic activity (Kumar, 1989; Seth, 2000; Kar *et al.,* 2008; Begum *et al.,* 2009) [1, 2, 3, 4] and presently it has been assumed that large proportions for aquatic life including fishes are adversely affected from the same. It is a high concern that heavy metal intoxication cause deleterious effect in different fish species such as enzyme inactivation, life span reduction, RBC and WBC surface shrinkage, mitochondrial dysfunction, genetic material breakdown, immunological dysfunction, alterations in haematological organization etc. Heavy metal pollution is considered as the burning issue due to its high solubility, persistence and biomagnifications in water which cause serious threat to aquatic ecosystem (Shukla and Tripathi, 2012) [5].

Heavy metals probably exert their toxic effect on fish by reacting with the mucous on the surface of the gills causing precipitation, coagulation and thus interfere with the normal gaseous exchange (Carpenter, 1927) [6]. The toxic effects of heavy metals on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body system (Dimitrova *et al*., 1944) [7]. The sub-lethal toxicity of heavy metals is known to produce haematological and neurological affects on fishes.

The uptakes as well as contamination of mercury by aquatic organism from water and sediment are influenced by various environmental factors including temperature, salinity, pH, dissolved oxygen, alkalinity, hardness, dissolve organic matter etc. Fish is one of the most potent victims of such contamination in water among other the aquatic fauna.

Heavy metals enter in to the fish body via contaminated water or food thereby causing severe pathological, immunological and behavioral alteration. Mercury is accumulated mostly in gill, liver, intestine and kidney causing severe effect on the fish.

The present investigation is an attempt to know the LC_{50} concentration of mercuric chloride on a model fish organism *Heteropneustes fossilis* and to study toxicity of mercuric chloride as a function of time on haematological alteration at sub-lethal concentration. The behaviour of the fish has also been aimed to study at different concentration of mercuric chloride.

2. Materials and Methods

2.1 Model Organism: The study has been carried out on *Heteropneustes fossilis* which is a common stinging catfish species has a wide natural distribution, distributed in the ponds, ditches, swamps, marshes, wetlands (beels) and also in muddy rivers of Assam. It posses accessory respiratory organ which makes it capable for breathing in atmospheric air. *H. fossilis* are common throughout the plains of India (including Andaman Island), Bangladesh, Pakistan, Myanmar, Nepal, Srilanka, Laos and Thailand. In Assam it is locally known as Singee. *Heteropneustes fossilis* has been selected as model organism as it is a live fish and well equipped with environmental condition of the region. Moreover, Das *et al.*, 2015 [8] study suggested that the general well-being and growth of the fish is good in the present environmental condition and geographical position.

2.2. Collection of Fishes: Live, healthy and disease free fishes (wt. 25-50 gram and length 7-15 cm) were collected from local fish market, Pandu, Guwahati-12 and bought to the laboratory without any mechanical injury. Fishes were treated with 0.2% $KMnO_4$ solution for 2-3 minutes to avoid any microbial infections and acclimatized in laboratory condition.

2.3. Acclimatization: The freshly collected fishes were kept in normal glass aquarium of 50 litter water capacity and acclimatized in laboratory condition for a period of 10-15 days at normal photoperiod to observe the visible pathological changes or symptoms.

The fishes were fed daily with commercial food during the period of acclimatization. The fecal matters and other waste products were washed off daily to reduce ammonia content in water by renewing a major portion of water every day. After 10-15 days, the acclimatized fishes were divided into two groups i.e. control group and experimental group. Some fishes were kept in the control condition throughout the period of experiment and some fishes were used for the experiment.

Control group consists of 10 fishes and treated group consists of 80 fishes. Treated fishes were exposed to different concentration of mercuric chloride to get the LC_{50} value and as well as to study behavior.

2.4. Preparation of experimental doses: Different concentration of mercuric chloride i.e. 200 mg/l, 100mg/l, 50mg/l, 25 mg/l, 1 mg/l, 0.16 mg/l, 0.14mg/l, 0.12mg/l, 0.10 mg/l, 0.08mg/l, 0.06 mg/l, 0.05 mg/l, 0.04mg/l, 0.03 mg/l, 0.02 mg/l, 0.01 mg/l and 0.001 mg/l were made for the experimental procedure to get LC_{50} value and behavioural study.

2.5. LC_{50} determination: The concentration at which 50% death of fish occur in 96 hours is considered as LC_{50} value.

2.6. Haematological study: After the determination of LC_{50}, the haematological study was performed at sub lethal concentration of LC_{50} ($1/5^{th}$ of LC_{50} concentration). The different haematological parameters were studied after an interval of 24hrs, 48hrs, 72hrs, and 96hrs. After exposure at sub lethal concentration, bloods of the fishes were collected from the heart and caudal peduncle of anesthetized fish. TLC was studied by using Neubauer's chamber (Daice and Lewis, 1977) [9]; DLC by Daice and Lewis, 1977 [9] method using Leishmann stain and Hb% was studied by using a haemometer. The physicochemical parameters were examined as per A.P.H.A., 1988 [10] and N.E.E.R.I., 1989 [11] at Departmental laboratory of Pandu College.

3. Results

3.1. Determination of LC_{50}

The concentration at which 50% death of fish occur for the exposure of 96 hours of toxicants is considered as LC_{50} value which is found to be 0.10 mg/l in this present investigation. For determination of LC_{50} concentration, the experimental doses of mercuric chloride are found to be active from 0.02 mg/l to 0.16 mg/l as only 1 fish dies at 0.02 mg/l concentration and It has been observed that 0.16 mg/l concentration of mercuric chloride can be considered as the minimum lethal concentration where 100% death of fish are observed. There are 8 aquariums of 25 litter water each with 10 fishes in every aquarium. A control aquarium is also maintained with 10 fishes. The entire aquariums are under proper aeration to maintain optimum oxygen level.

Table 1: Table to determine LC_{50} with concentration of mercuric chloride

Number of exposed	Number of survived	Number of respond	Concentration(mg/l)
10	10	0	00
10	9	1	0.02
10	8	2	0.04
10	7	3	0.06
10	6	4	0.08
10	5	5	0.10
10	3	7	0.12
10	1	9	0.14
10	0	10	0.16

3.2. Haematological Studies

After determination of LC_{50}, the fishes are treated at sub lethal concentration of LC_{50} ($1/5^{th}$ of LC_{50} concentration), which in this investigation is 0.02 mg/l. The fish samples are collected after the day of exposure from the aquarium at an interval of 24, 48, 72 and 96 hours for haematological studies. Haemoglobin found in control experimental fishes showed an average value of 5.77%. The fishes that are exposed to sub lethal concentration of mercuric chloride shows a beautiful decline trend of haemoglobin concentration from 5.18% to 2.74% as the time of exposure increases (Table-2). It is worth mentioning here is that the values of Hb% after treatment showed a significant decrease when compared to control.

The erythrocyte count of controlled aquarium fishes showed a mean value of 2.02×10^6 mm^3. The fishes exposed to sub-lethal concentration of mercuric chloride showed a decline trend as the exposure time increases. The mean values of RBC are found to be 1.07×10^6, 0.99×10^6, 0.51×10^6, 0.41×10^6 mm^3

after a time interval of treatment 24hrs, 48hrs, 72hrs and 96hrs respectively (Table-2). The present study also reveals that RBC is significant decreased as compared to control.

The results of total count of white blood cells revealed that the blood of the controlled aquarium fishes shows a mean value of 15.02×10^3 mm^3. The fishes exposed to sub lethal concentration reveals a beautiful trend where an increase trend is observed and shoots up to 28.97×10^3 mm^3 at an concentration of 48 hours of exposure through 26.37×10^3 mm^3 at 24 hours of exposure and decline steadily from 22.40×10^3 mm^3 at 72 hours of exposure to 16.47×10^3 mm^3 at 96 hours of mercuric chloride exposure. The experiment also suggests that the measure of WBC is always found to be the minimum in controlled aquarium.

Table 2: Effects of sub-lethal concentration of mercuric chloride on heamatological parameters of *H. fossilis* at different exposure time period at sub lethal concentration in experimental aquariums

Parameters	Control	24hr (treatment)	48hr (treatment)	72hr (treatment)	96hr (treatment)
Hb percentage (g/100ml)	5.77	5.18	4.44	3.10	2.74
Total RBC count (mm^3)	2.02×10^6	1.07×10^6	0.99×10^6	0.51×10^6	0.41×10^6
Total WBC count (mm^3)	15.02×10^3	26.37×10^3	28.97×10^3	22.40×10^3	16.47×10^3

The results of differential Leucocytes Count (DLC) are depicted in Table-3. The time of exposure play a key role in DLC of the blood. The results suggests that Basophil shows a increasing trend as the time of exposure increases with a reverse phenomena observed in Neutrophil and Monocytes where decreasing trend is observed. Lymphocyte is observed to be high at 48 hours of exposure of mercuric chloride.

Table 3: Differential Leucocytes Count (in % near to whole number) experimental aquariums at sub lethal concentration

White blood cell types	Controlled condition	24hr (treatment)	48hr (treatment)	72hr (treatment)	96hr (treatment)
Basophil	23	26	28	31	35
Eosinophil	4	5	3	4	4
Neutrophil	12	9	8	8	6
Monocytes	14	11	9	8	8
Lymphocytes	47	49	52	49	47

3.3. Water Chemistry

The water chemistry of the aquariums in the present study is depicted in the Table-4. The most important parameters like pH, Temperature, DO, Free CO_2, Total Alkalinity, Total Hardness which are important for the fish to grow are considered in the present investigation. The water has been monitored in the studied aquariums so that there is a minimal effect or no effect in the life of the fish. However, Total Alkalinity and temperature measures a little more in mercury treated aquarium on the other hand temperature, pH, Free CO_2 are found to be a little lesser than acclimatised and controlled aquariums (Table -4).

Table 4: Physicochemical parameters of water in experimental aquariums

Parameters	Average±Standard deviation		
	Acclimatized aquarium	Controlled Aquarium	$HgCl_2$ treated Aquarium
P^H	6.83±0.25	6.89±0.23	6.96±0.23
Temperature(in ºC)	25-28ºC	28-30ºC	29-32ºC
D.O. in mg/l	7.96±1.66	7.83±1.42	6.86±1.20
Free CO_2 (mg/l)	7.12±1.23	7.62±1.54	7.92±1.60
Total alkalinity as $CaCO_3$ in mg/l	60.5±2.96	62±3.13	63±2.56
Total hardness as $CaCO_3$ in mg/l	57±2.97	58.20±2.31	57±3.13

3.4. Behavioural changes

A behavioral study is also conducted in the present investigation. After the exposure of mercuric chloride to the acclimatized fishes, the pattern of behavioral change is observed without remarkable morphological changes. The weight of the fishes does not show marked difference amongst the treated fish and also to that of controlled fishes.

The behavior of the fish is studied from very low concentration (0.001 mg/l) to above lethal concentration (200 mg/l). However, it has been observed that at low concentration of 0.001mg/l, the treated fishes do not show much behavioral changes; but at LC_{50} concentration (0.1 mg/l), half of the treated fishes were seen irritated, uncomfortable, becomes restlessness and at minimum lethal concentration (0.16 mg/l), the treated fishes are seen frequent surfacing whereas at acute concentration of 200mg/l they try to jump out of water for breathing and ultimately die by showing a special circling behavior, just the fishes are added to the toxicant. It is also noteworthy here is that fishes can survive only for 5-6 minutes after exposure of 200 mg/l acute dose.

At sub lethal concentration, the fishes shows excitation or hyperactivity, rapid movement, high rate of mucus secretion, loss of balance, circling or spiral movement, occasional jerking of the body and tendency to remain on the surface. It is interesting to note that the change of behavior occur as the time of exposure changes (Table-5). The study reveals that 48 hours of exposure of mercuric chloride is important time where fishes show maximum surfacing with the highest hyperactivity. At 24 hours tail as well as opercula movement is found to be the maximum. Loss of balance and circling behavior is observed from 72 hours of exposure of mercuric chloride at sub lethal concentration.

Table 5: Behavioral response observed for mercuric chloride toxicant at sub lethal concentration (0.02mg/l) Where - (nil), + (less), + + (moderate), + + + (prominent)

Activity	Time of exposure				
	Control	24 hours	48 hours	72 hours	96 hours
Surfacing	+	+ +	+ + +	+ +	+
Loss of balance	-	-	-	+	+
Opercular movement	+ +	+ + +	+ +	+ +	+
Mucous secretion	+	+ +	+ +	+ + +	+ +
Hyperactivity	+	+ +	+ + +	+ +	-
Circling	-	-	-	+ +	+
Tail movement	+ +	+ + +	+ +	+ +	+

4. Discussion

The LC_{50} concentration in the present study after an exposure of mercuric chloride for 96 hours is found to be 0.10 mg/l, which is considered as a low concentration indicating the adverse effect of the toxicant used in the study. The present study also reveals that the exposure of fish to the sub-lethal concentration (0.02mg/l) for 24 hours, 48 hours, 72 hours and 96 hours caused noteworthy variation in haematological parameters in *H fossilis.* Haemoglobin and RBC are reduced as the time of exposure is increases (Table-2). Panigrahi and Mishra, 1978 [12] also observed reduction of haemoglobin percentage and RBC count of the fish *Anabus scandens* treated with mercury. Dutta *et al.*, 2015 [13] and Pandit and Sharma, 2019 [14] also reported similar phenomenon of reduction of haemoglobin percentage and RBC count of the fish *Channa punctatus* treated with Lead. The present study also suggests a reduction of haemoglobin percentage almost to 50% after an exposure of 96 hours of sub lethal concentration of mercuric chloride, thus, leading to anaemia. Significant reduction of RBC and haemoglobin content in fish exposed to different heavy metals has been reported previously by Goel and Sharma, 1987 [15]; Dutta *et al.*, 2015 [13]; Dutta *et al.*, 2016 [16].

White blood cells play a major role in the defense mechanism of the fish and consist of Lymphocytes, Monocytes, Basophil, Eosinophil and Neutrophil. During exposure of mercuric chloride, higher WBC indicates disturbing state due to infection of body tissues, severe physical stress as well as other associated factors. WBC are found to be increased following mercuric chloride exposure till 72 hours of exposure and then steadily decreases to a certain extent (Table-2), which corroborates with the result of Dutta *et al.*, 2015 [13]. Misra and Shrivastava, 1980 [17] also reported an increase in leucocytes count when they exposed fishes to heavy metals. Leukocyte counts showed greater and quite different pattern of change with treated fish with mercuric chloride when compared with the leukocyte counts of the control group. The increase in WBC count observed in the present study could be attributed to stimulation in the immune system in response to tissue damage caused by mercuric chloride (Gill and Pant, 1985) [18]. Gill and Pant, 1985 [18] also reported that the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage.

Differential Leucocytes Count (DLC) shows an increasing trend as the exposure of mercuric chloride increases. Specially Lymphocytes shows an increasing trend from lower to higher as a function of exposure time with more or less similar trend with Basophil, where the highest peak is observed at an exposure time of 48 hours and then decline slowly. However, Eosinophil, Neutrophil and Monocytes show a reverse phenomenon where the number of counts decreases steadily.

The physicochemical parameters in all experimental aquariums are maintaining at the normal range (Table-4) so that there is no adverse effect on the fish. Moreover, the experimental fish is a live fish and therefore the effect of DO and FCO_2 concentration is minimal.

The various behavioral changes like restlessness, abnormal swimming behavior, vigorous jerks of body, loss of balance, surfacing, encircling, mucus secretion and anorexia observed in *Heteropneustes fossilis* indicates the effect of the toxicity of mercuric chloride. This type of result is also observed by Chandra, 2008 [19] in the same fish species exposed to various pesticides.

5. Conclusion

The haematological parameters in a fish are reflection of the physiochemical condition of its habitat including toxic ingredients. In recent years haematological variables have been used more to determine the sub lethal concentrations of pollutants in a reverse way. Behavioral changes as a result of stress are further accepted as the most sensitive indication of potential toxic effects. Most importantly, from the study it can be concluded that fishes are one of the most potent victims of heavy metals among the aquatic fauna and serve as a good indicator of aquatic pollution. Besides the pathological changes in fishes, another most devastating problem is bioaccumulation and with time, it can enter the food chain as fishes are considered as a good source of animal protein harming human being too.

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