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ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2017, 6(5): 88-95

Toxicological Assessment of Barium Carbonate on Behavioral and Hematological Parameters in *Channa punctatus*

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ABSTRACT

Barium and its compounds are known for their toxic nature. In the present study barium carbonate ($BaCO_3$), a known rodenticide has been assessed for its toxicological effects on Channa punctatus. Behavioral as well as hematological parameters including body colour, movements, food intake and mucus secretion were studied. Fingerlings of Channa punctatus were observed for one week and the results revealed cell membrane damage and nuclear shrinkage in RBCs following two different concentration treatments of barium carbonate. A dose and duration dependent toxicological effect was seen. Conclusively, the present study confirms the toxic nature of barium carbonate to Channa punctatus as revealed by the hematological as well as behavioral end points. As only a few studies have been done on the toxic aspects of $BaCO_3$ on different animal models, further short term studies should be conducted so as to complete the toxic profile of barium carbonate.

Keywords: Barium carbonate, Toxicity, *Channa punctatus*, Blood, Nucleus, Fish

INTRODUCTION

Barium (Ba) with CAS number 513-77-9 and atomic number 56 is a soft silvery metallic earth metal. Because of its reactive nature, Ba is never found in nature as a free element. Various compounds of barium found in nature are barium chloride ($BaCl_2$), barium nitrate [Ba $(NO_3)_2$] and barium hydroxide ($BaOH_2$). Barium compounds are relatively insoluble in water but dissolve in the stomach thus showing harmful health effects. Taking large amounts of barium compounds can cause changes in heart rhythm and paralysis in humans. Barium nitrate has been found to be toxic when ingested or inhaled [1-4]. Symptoms of poisoning include tightness of muscles, vomiting, diarrhea, abdominal pain, and muscular tremors. Barium also acts as a potent muscle poison. Most of toxic effects of barium results from its ability to cause a profound reduction in serum potassium together with an increase in intracellular potassium. Aqueous solutions of barium hydroxide and barium oxide are strongly alkaline and cause significant ocular burns and skin irritation. The carbonate and sulfate of barium are irritating to the mucous membranes of the upper airways and can also cause irritation to the skin and eyes. Barium carbonate ($BaCO_3$) procured from witherite, is a chemical compound that is commonly used as rat poison. Some studies have revealed the toxicity of barium carbonate [5-9].

For toxicological studies, blood provides an ideal medium. Blood is the most abundant and important body fluid. The composition of blood reflects the total physiological condition of the body. The hematological parameters have been considered as diagnostic indices of pathological conditions. Fish blood can provide a valuable tool in detecting physiological changes taking place in aquatic animals [10]. Fishes are highly sensitive to exposure of various pollutants. *Channa punctatus,* also known as spotted snakehead is found in India, Sri Lanka, Nepal, Afghanistan, Bangladesh, Myanmar, and China [11]. Its habitat includes ponds, swamps, and brackish water systems. *Channa punctatus* are carnivorous voracious feeders and have been used as a model for assessing the toxicity of various chemical compounds [12-18].

Heavy metals are one of the major pollutants, which are present in small quantities in the earth's crust. Aquatic environment is constantly polluted with heavy metals and various chemical compounds by various natural processes as well as anthropogenic interferences [11,19-22]. Heavy metals cause harmful effect on RBCs life span, hemoglobin surface area and cause alterations in hematological parameters of different fish species. Fishes are one of the potent

victims of such contaminated water among the aquatic fauna and serve as a good indicator of aquatic pollution [23]. Likewise, Ba compounds are also prevalent in different ecosystems which can affect the habitat of *Channa punctatus*. Only a few papers are available on the reports of toxicity of barium compounds on fishes. Thus, in the present paper, an attempt has been made to access the toxicity of barium carbonate using *Channa punctatus* as a model on various behavioral and hematological parameters.

MATERIALS AND METHODS

Materials

In the present study, *Channa punctatus* has been used as a model to access the toxicity of $BaCO_3$ on blood parameters and behavior. For this study, live specimens of fingerlings of *Channa punctatus* with an average length of 10 cm were collected from local fish market of Amritsar. The fingerlings were transported to the lab in polythene bags containing O_2 saturated water. Both Leishman stain and Methylene blue (Qualigens) were used for staining the slides. Slides were observed at 100X magnification under light microscope (Olympus CH 21) using oil immersion.

Methods

Preparation of exposure concentrations

Two different concentrations of $BaCO_3$ were prepared by dissolving 0.25 g and 0.5 g of analytical grade barium carbonate in 5 L distilled water (as per the volume of the tubs used in the experiment). These concentrations of barium carbonate (50 ppm and 100 ppm) were used to assess the toxicity parameters in *Channa punctatus* and were compared with the control fingerlings (Figure 1).

Figure 1 Control fingerlings in a single plastic tub under observation

Figure 2 A single fingerling used in the experiment (Avg. Length=10cm)

Experimental setup and processing of fingerlings

For the present experiment, 6 plastic tubs of volume 8 L each were set in the lab. Twelve fingerlings were put in tubs (2 per tub) to assess the exposure results. Two different concentrations of $BaCO_3$ were made by dissolving appropriate amount of analytical grade barium carbonate in distilled water. After exposing to different concentrations for different durations (for 1-6 days), 2 fingerlings were removed for procuring blood. Fingerlings in tubs 1, 2 and 3 were exposed to a concentration of 50 ppm whereas fingerlings in tub 4, 5 and 6 were exposed to a concentration of 100 ppm $BaCO_3$. Fingerlings were provided sufficient food (egg white) and following parameters were observed over a time period of 1 week (Figure 2).

1. Body colour changes

2. Fish movement

3. Food intake

4. Mucus secretion

A score (out of 5) was given to the fingerlings in tub 3 and 6 on each day following an exposure to 50 and 100 ppm $BaCO_3$ respectively (Table 1).

Preparation of blood smears

Live and healthy fingerlings were exposed to different concentrations of barium carbonate for 6 days. After 2 days, one fingerling from tub 1 and 4 were taken out and blood was drawn out from the caudal vein using a 2-ml syringe. Two replicate smears were made for each fish exposed at a given concentration. Blood smear slides were allowed to dry for few seconds and then were stained with Leishman and Methylene blue for 60 seconds. Then each slide was washed with distilled water and allowed to dry for 10 minutes at room temperature and studied under light microscope at 100X magnification. The same procedure was followed for tub 2 and 5; and tub 3 and 6 at day 4 and 6, respectively for hematological parameters.

RESULTS

Blood is known as pathophysiological reflector of the animal body; Therefore, blood parameters are important in diagnosing the status of an animal exposed to toxicants. The foremost cause of toxicant exposure in fishes is anemia which causes injury to the RBCs thus leading to decrease in their oxygen carrying capacity. A significant reduction in RBC count, Hb% and increased WBC count are also observed. Decrease in Hb% can be either due to decrease in the rate of erythrocyte production or increase in the loss of these cells. In the present experiment, the following symptoms of behavioral changes and poisoning have been observed.

Body colour changes

The exposed fingerlings were kept in observation for 6 days and a regular record of body colour change was noted. It was seen that during the exposure to barium carbonate, the colour of fish became lighter with each passing day (Table 1). On the first day, the fingerlings were of dark grayish colour (scale 5), as per Table 1, and with each passing day, the colour changed to light gray (scale 4.7-1).

Table 1 Behavioral changes recorded in fingerlings of *Channa punctatus* with respect to duration of exposure (100 ppm $BaCO_3$)

Sr. No.	Parameters studied	Grades of behaviour					
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Body colour change	5	4.7	4.8	4.2	3	2
2	Fish movement	5	4.5	4.3	3.2	2	1
3	Food intake	5	4.8	4	2.8	2	1
4	Mucus secretion	1	1	2	3	4.7	5
All the grades are out of 5; 5 being the highest and 1 being the lowest grade							

Fish movement

Twelve fingerlings were kept in 6 tubs (2 per tub) keeping a regular record of fish movement following the exposure to barium carbonate from the very first day up to the 6th day. The recording of the fish movement was done at 9 am, 1 pm and 4 pm each day and it was seen that with time, the activity of fish was reduced (Table 1). Before exposure the movement of fish was more as compared to when it was exposed to the compound (Figure 3).

Food intake

The effect of barium carbonate toxicity was also seen on food intake preferences. With increasing duration of exposure, the fingerlings were seen to have lesser food intake as described in Table 1. Exposed fingerlings were observed to take normal food on 1st and 2nd day (scale 5) and it kept on decreasing by 3rd, 4th and 5th day to the minimum scale of 1 at last day of the week.

Mucus secretion

Mucus is known for its protective effect in various animals. In the experimental setup, it was seen that in the starting days of exposure there was a normal mucus secretion over the body and gills but as duration of exposure to barium carbonate increased, an increased mucus secretion was seen over the whole body and gills of fingerlings. This increased mucus secretion may be the protective method against the inflammatory response by the fish.

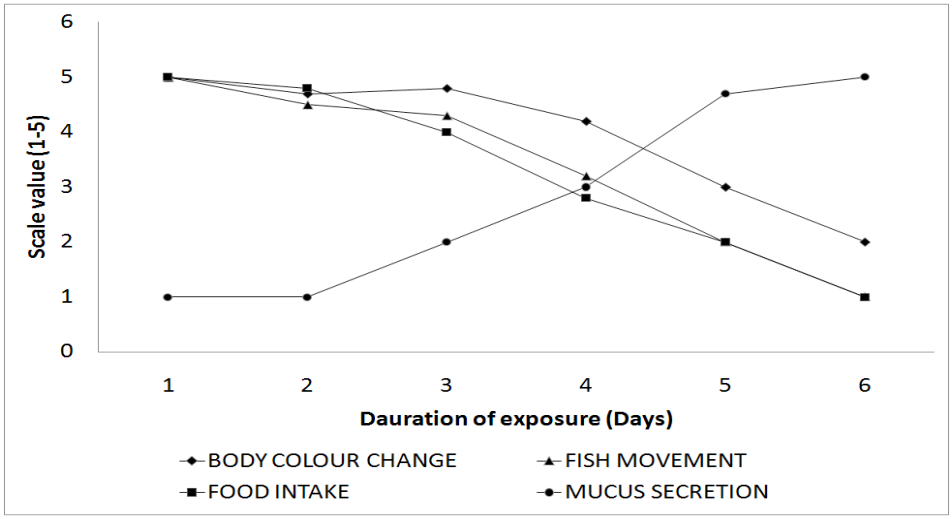

Figure 3 Different behavioral parameters in response to $BaCO_3$ exposure. All the values are on a scale of 5 (1 being the lowest and 5 being highest)

Hematological parameters

For the hematological parameters, 12 fingerlings were taken under the present study. Fingerlings were exposed to two different concentrations of barium carbonate (50 ppm and 100 ppm) and the blood smears were analyzed on 2nd, 4th and 6th day for any anomalies including cell membrane damage and nuclear damage in RBCs (Figure 4). On exposure to 50 ppm barium carbonate, no change in the cell membrane was observed on 1st and 2nd day of exposure. As the duration of exposure increased (4th day) cell membrane damage was seen in the slides (Figure 5; denoted 'B'). Following extended exposure to barium carbonate for 5th and 6th day, toxic effect on both nucleus and cell membrane was observed. Reduced nucleus and wrinkled cell membrane were observed (Table 2). On exposure to 100 ppm barium carbonate, minor cell membrane damage was observed on 2nd day. As duration of exposure increased (4th day) cell membrane damage and reduction in the size of nucleus was observed (Figure 6; denoted 'C'). Following extended exposure to barium carbonate for 5th and 6th day, nucleus was observed to be reduced in size and shrunken. A wrinkled cell membrane was also observed (Table 2 and Figure 7; denoted 'D'). All the above mentioned nuclear and cell membrane anomalies were not seen in control fingerlings as seen in Figure 4 (Control RBCs are denoted as 'A1').

Table 2 Blood cell changes in *Channa punctatus* with respect to duration of exposure and exposure concentration

$BaCO_3$ concentration	Duration of exposure		
	Day 2	Day 4	Day 6
50 ppm	No change	Cell membrane damage	Wrinkled membrane and reduced nucleus
100 ppm	Minor cell membrane damage	Cell membrane damage, nucleus reduced in size	Cell membrane wrinkled, nucleus shrinks

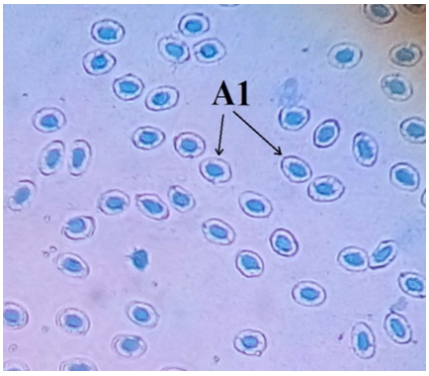

Figure 4 Blood smear of control fingerlings showing normal RBCs (A1)

Figure 5 RBCs of fingerling exposed to 50 ppm $BaCO_3$ at Day 4 (B)

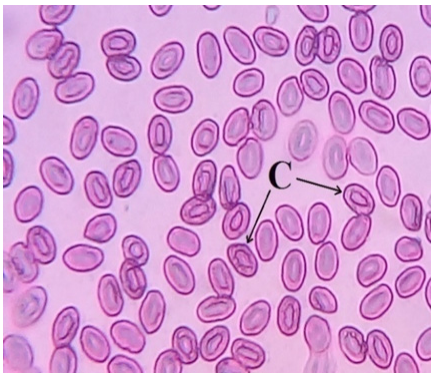

Figure 6 RBCs of fingerlings exposed to 100 ppm at Day 4 (C)

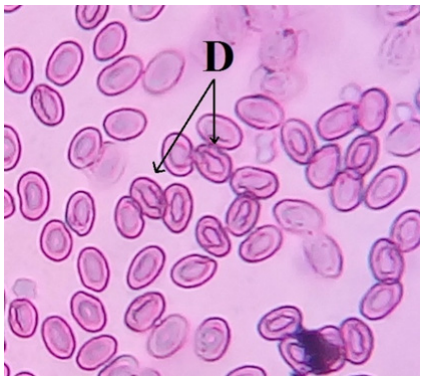

Figure 7 RBCs of fingerlings exposed to 100 ppm at Day 6 (D)

DISCUSSION

Behavioral parameters

In the present study, fingerlings of *Channa punctatus* were used as a model to assess the toxic effects of $BaCO_3$ at different sub lethal concentrations (50 ppm and 100 ppm). The study observed behavioral as well as hematological parameters for assessing the toxicity. Twelve fingerlings were put in tubs (2 per tub) and were exposed to $BaCO_3$ and the changes in body colour, movements, food intake and mucus secretion have been tabulated in Table 1. Body colour was found to be lighter with the increasing duration of exposure at both the concentrations. In another study, body colour change from normal to black was found during the lethal exposure of cypermethrin in Labeo rohita. The reason for this change may be the irritability of skin to $BaCO_3$. Similarly, the general fish movement in the tub was observed for 1 week and a remarkable decrease in the movement as compared to controls was recorded. A grade of 1 was given to the fingerlings following 100 ppm $BaCO_3$ exposure on 6^{th} day. A time dependent decrease in fish movement was seen (Figure 3). Food intake was also observed to be reduced along with the fish movement which could be the result of the induction of toxic mechanisms following the $BaCO_3$ exposure. A very characteristic symptom of poisoning with a local effect is the abundant discharge of mucus at the gills and on the skin covering over the pupil of eyes. Mucus on the skin in these cases plays an important role in the protection of fishes from the harmful toxic effects of chemicals. At lower concentrations, however changes in behavior were not as conspicuous. On contrary, the mucus secretion was found to be increased. A change from grade 1 to grade 5 was recorded in 6 days of observation following 100 ppm $BaCO_3$ exposure (Table 1 and Figure 3).

Hematological parameters

The exposure of fish to sub lethal concentrations of barium carbonate for 2, 4 and 6 days caused significant alterations in blood cell parameters in *Channa punctatus*. Marked increase in the parameters was observed in a duration and dose dependent manner. In our study, cell membrane damage was observed to start on 4^{th} day at 50 ppm $BaCO_3$ exposure whereas the same has been observed on day 2 following 100 ppm exposure (Table 2) which confirms a dose dependent toxic effect. In line with our study, Alkesh and Bharat [10] revealed a reduction in fish RBC count. It was presumed that reduction of RBC might actually be due to inhibition of RBC production with destruction of previously present RBC on exposure of fish to the pollutant. Similarly, round and swollen RBCs (spherocytes) were reported by Sawhney and Johal [24]. According to Sawhney and Johal [24] and Massar, et al. [25], alterations in morphology of erythrocytes in the blood of fish are associated with pesticide toxicity as well as heavy metal pollution. Therefore, various alterations induced by barium carbonate in the current study can be correlated with altered surface membrane area to volume ratio of cell. The same has also been reported by Naskar, et al. [26] in Clarias batrachus due to aluminium toxicity. According to Tanka and Nakai [27] the normal biconcave shape of rat erythrocytes changes to spherocytes on exposure to mercuric chloride.

CONCLUSION

Barium carbonate ($BaCO_3$) is a known toxic chemical but only a few toxicological studies have been conducted using this chemical on animal models. In the present study, the toxicity of $BaCO_3$ was assessed using *Channa punctatus* as a model animal. We formulated two sub lethal concentrations (50 and 100 ppm) of $BaCO_3$ and fingerlings were exposed and were observed for a week for behavioral as well as hematological parameters. The results revealed its toxic nature as shown by anomalies in blood cell parameters including cell membrane damage and nucleus shrinkage in RBCs. Conclusively the present study confirms the toxicity of $BaCO_3$ on *Channa punctatus* but further short term toxicological studies on different organs in *Channa punctatus* are also required to complete the toxic profile of barium carbonate.

ACKNOWLEDGEMENTS

The authors are sincerely thankful to Department of Zoology, Khalsa College Amritsar and Dr. Jasjit Kaur Randhawa (Head) for providing necessary infrastructure to carry out the present study.

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