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# Calcium carbonate supplementation causes preventive approach to toxic effects of mercuric chloride on metabolic regulation in liver of *C. punctata*

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## ABSTRACT

*Mercury (Hg) shows the toxic effects in the environment although the etiology is not well characterized and the prevention of toxic effects induced by Hg is an important aspect of metabolic regulation in organisms. Channa punctata, a variety of species of fish was used in this study and the role of calcium carbonate on cholesterol, triglyceride and protein level in liver induced by $HgCl_2$ was adopted. Fish were exposed to 1 and 10 µM of $HgCl_2$ for 1h and cholesterol and triglyceride levels in excised liver were enhanced in response to $HgCl_2$ when compared to respective controls however the effects were more pronounced for 1 µM concentration. Similar stimulatory effects on protein contents were demonstrated whenever they were exposed to $HgCl_2$ (1 and 10 µM) and higher proteins were recorded for 10 µM concentration. The results indicate that $HgCl_2$ causes severe toxic effects enhancing the above parameters. To clarify the role of $CaCO_3$ on prevention of these effects, fish were treated with different concentrations (100 µM and 1 mM) of $CaCO_3$ and $CaCO_3$ + $HgCl_2$. Cholesterol and triglyceride in liver were effectively reduced with $CaCO_3$ (1 mM) + $HgCl_2$ (10 µM) and $CaCO_3$ (100 µM) + $HgCl_2$ (1 µM) while 100 µM of $CaCO_3$ potentially reduced the effects of $HgCl_2$. Although $CaCO_3$ was shown to reduce protein content effectively, however 100 µM concentrations have been found to inhibit the effects of $HgCl_2$ preferentially. Our findings suggest that calcium carbonate might be involved in prevention of the toxic effects of Hg and may contribute to the survival process of this species.*

***Key words**: Channa punctate, toxic effects, liver, metabolic regulation and mercuric chloride*

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## I. Introduction

Mercury (Hg) is recognized to be an important pollutant and its compounds exist in the biosphere and are bioaccumulative and toxic. Consequently, mercury contaminations cause a serious risk to humans and ecosystems (Shastri and Diwekar, 2008). In aquatic environments, this heavy element has been



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demonstrated to be as a metallic or elemental form, inorganic compounds or organic compounds (Black *et al*., 2007). These forms have been found to have toxicological effect, metabolic fate and biochemical actions. Although organic mercury compounds are more toxic in the biosphere, inorganic compounds are the most common form of mercury and released in the aquatic environment by industries. These inorganic mercury compounds have been found to have a more potential role on fish tissue (Oliveira Ribeiro *et al*., 1996) and the mechanism of prevention of the toxic effects caused by mercury is not clarified well. Foreign toxic compounds are discharged daily into water bodies and are believed to induce reactive oxygen species (ROS) which cause cell and tissue damage (Elia *et al*., 2003). It has been demonstrated that Hg toxicity involves the formation of ROS and the antioxidant defense system has been shown to be altered markedly resulting oxidative damage such as lipid peroxidation and cell death (Elia *et al*., 2003 and Verlecar *et al*., 2008). For characterization of fish diseases, various pathologic processes have been demonstrated by investigator (Banerjee *et al*., 1999). Liver is a major tissue where detoxification is observed. Therefore, identification of the toxic effects and its detoxification are essential parameter in biotransformation of the foreign toxic substances in this tissue of the organisms.

*Channa punctata* (Taki fish) is strong and survive in adverse environment; however, the survival of these fish is impaired by the environment. Therefore, it is an important issue to determine the strategy for the prevention of the toxic effects of Hg. Although several lines of evidences for the prevention of heavy metal toxicity have been demonstrated, however the regulatory mechanism of these approaches is not well characterized. Therefore, the current study has been undertaken to find the toxic effects of Hg and identify the prevention of the toxic effects particularly the role of calcium carbonate ($CaCO_3$) on cholesterol, triglyceride and protein content in liver of this species of fish. *C. punctata* is a fresh water fish and virtually found in haor, bil and river of Bangladesh. They are much energetic and survive in the adverse environment for long time. For human being, the fish are used in the diet as a major source of protein. It is assumed that the increased activity of the sympathetic nerves in this fish may contribute to the higher energy content.

Peripheral tissue metabolism has been shown to be influenced by environmental and chemical stimuli; however, endogenous auto regulation of these processes of all species is a common biological phenomenon. Degradation of biomolecules and its biosynthesis is the characteristic feature of metabolic processes. Liver plays a vital role in metabolic coordination and regulation of all the peripheral tissues. Both environmental and chemical stimuli have been found to cause metabolic alterations in this tissue. Although fish are exposed to various environmental stimuli, they exhibit their efforts to maintain the homeostasis of body. During environmental adverse condition, the liver of this species might be associated on its metabolite regulation so that they can survive in the atmosphere. To survive in the atmosphere affected by toxic mercury, the biological role of liver on adaptive response on metabolic regulation is not defined. As a metabolic organ, liver plays a major role in detoxification of foreign molecules. Therefore, the organ may also serve as a regulatory area to the sensitivity of toxic substances. The present study has been adopted to determine the acute toxicity of mercuric chloride and to evaluate the oxidative stress responses particularly on cholesterol, triglyceride and protein content in liver of *C. punctata* and to find the strategy for the prevention of the toxic effect of mercuric chloride.

## II. Materials and Methods

The experiment was conducted during August 2011 to December 2014. Methodology involved with this experiment discussed below.

**Fish:** *C. punctatus* weighing 50 g to 60 g were used and maintained in normal water with ambient temperature (25.0 ± 1 $^{o}$C). They are very strong and survive in the environment for prolonged period. In the day of experiment, exposure of mercuric chloride and calcium carbonate were given to the different groups of fish in small plastic pots for 1h period with full aeration and with free access of water. After the treatment, fish were quickly decapitated and liver was sampled carefully and taken



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weight by digital balance (Chyo, JL-180, China) and kept at –20 °C. Control fish were similarly used for sampling of tissue except giving mercuric chloride or calcium carbonate exposure.

**Mercury treatment:** To examine the role of heavy toxic element on the regulation of metabolic activity involving the amount of protein, triglyceride and cholesterol in liver, groups of fish were used with different concentrations of mercuric chloride (1 and 10 µM) ($HgCl_2$, BDH Chemical Ltd.) in water (600 mL) for 1h. After the treatment, fish were quickly decapitated and liver was sampled carefully and taken weight by digital balance. Cholesterol, triglyceride and protein contents in extracts of liver of fish treated with $HgCl_2$ were determined.

**Calcium carbonate treatment:** Calcium carbonate (100 µM and 1 mM) was prepared with water, dissolved with concentrated HCl and was made pH 7.0 with diluted NaOH. The following groups of fish treated with $CaCO_3$ (100 µM and 1 mM) (600 mL) for 1h were used to examine the role of calcium carbonate on metabolic regulation in liver induced by $HgCl_2$: a) Control b) $HgCl_2$ (1 µM) c) $HgCl_2$ (10 µM) d) $CaCO_3$ (100 µM) e) $CaCO_3$ (1 mM) f) $HgCl_2$ (1 µM) + $CaCO_3$ (100 µM) g) $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM). The groups of fish were treated with $HgCl_2$ and $CaCO_3$ in ambient temperature for determination of different parameters. The liver was sampled after the treatment similarly as mentioned above and cholesterol, triglyceride and protein contents in each liver extract were determined.

**Assay of tissue cholesterol, triglyceride and protein content:** Cholesterol content in liver was determined by using the method of Liebermann-Barchard reaction (Kenny, 1952). For assay of cholesterol, 0.5 mL of crude extract was taken to test tubes and 10 mL of ethanol-ether mixture (3:1) were added. The test tubes were shaken vigorously and the contents were taken to centrifuge tubes and were centrifuged for 15 min at 8000 rpm. The supernatants were transferred to new glass tubes and evaporated to dryness in a water bath. After evaporation, 5 mL of chloroform were added to dissolve the residue and 2 mL of acetic anhydride-$H_2SO_4$ mixture (20 mL of acetic anhydride and 1 mL of concentrated $H_2SO_4$) were given, mixed and allowed to stand in dark at 25 °C for 20 min to develop the color. The spectrophotometer reading was taken at 680 nm against the blank. Cholesterol content was measured with the help of standard solution of cholesterol (20 mg/100 mL in chloroform) where 2.5 mL of standard solution was taken in test tubes and 2.5 mL of chloroform were mixed and followed the same procedure. For blank, only 5 mL of chloroform and 2 mL of acetic anhydride-$H_2SO_4$ mixture were used. The amount of cholesterol was expressed as mg/100 g of tissue weight. Triglyceride content in liver of different groups of fish was measured quantitatively by LABKIT (Triglycerides kits), Crest Biosystems, Bambolim Complex Post Office, Goa - 403 202, INDIA. For assay of triglyceride, 100 µL of crude liver sample were used. Tissues were homogenized with pre-cooled water and were centrifuged at 8000 rpm for 10 min. The supernatants from each tissue homogenate were used as crude extract for assay of protein by using 50 µL extract. The protein content in tissue was determined by the procedure of Lowry *et al.* (1951). Briefly, alkaline solution was prepared by mixing 50 mL of alkaline $Na_2CO_3$ solution (2% $Na_2CO_3$ in 0.1N NaOH) and 1.0 mL of copper-sodium potassium tartarate solution (1 g sodium potassium tartarate and 0.5 g $CuSO_4$. $5H_2O$ were dissolved in 100 mL distilled water). Fifty micro liters of tissue extract was taken to the test tube and made up to 1 mL with distilled water. For blank, 1 ml water was used in place of tissue extract. Five milliliters of alkaline solution was added to each tube and mixed well. The tubes were allowed to stand for 10 min at room temperature and 0.5 mL of diluted FCR (Commercial FCR was diluted with equal volume of water) was added and mixed well. After 30 min, the absorbance was taken at 650 nm against the blank. The protein content in each tissue was calculated from the standard graph of bovine albumin (1 mg $mL^{-1}$) and is expressed as mg/100 g of tissue weight.

**Statistical analysis:** Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by paired *t*-test using SPSS software.



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## III. Results

### Effects of mercuric chloride on cholesterol level in liver

Mercuric chloride ($HgCl_2$) is toxic to the living organisms. Exposure of higher concentration of mercury in water causes severe effects in fish and might be involved in the impairment of metabolic activities in cellular level. Liver is the major area where biotransformation of foreign toxic substances occurs. The uptake and detoxification of mercury in liver is an important aspect in liver metabolism. The cellular uptake of mercury may impair lipid metabolism. To clarify whether $HgCl_2$ affects cholesterol level in liver, groups of fish (*C. punctata*) were exposed to $HgCl_2$ (1 and 10 µM). After the treatment, liver was excised and cholesterol content in liver was determined. Control fish were similarly used except $HgCl_2$ treatment. As shown in Figure 01, the average cholesterol content in liver of fish exposed to 1 µM concentration of $HgCl_2$ was 1678.42 ± 288.44 mg while for the control fish, the value was 145.73 ± 17.95 mg/100 g of tissue weight. The results show that cholesterol content in liver was increased significantly (11.5 folds) ($p < 0.001$) when compared to the liver of control fish. On the contrary, fish exposed to different concentrations of $HgCl_2$ (10 µM) for 1h had 911.14 ± 194.75 mg of cholesterol. Mercuric chloride causes the synthesis of cholesterol significantly ($p < 0.001$) (6.2 folds) when compared to the liver of control fish, however 1 µM concentration of mercuric chloride was found to be involved in higher synthesis of cholesterol than 10 µM concentration. The results appear to indicate that mercuric chloride is toxic compound and might be involved in causing the chemical and environmental stresses seemed to cause the synthesis of cholesterol and induce lipogenesis in liver.

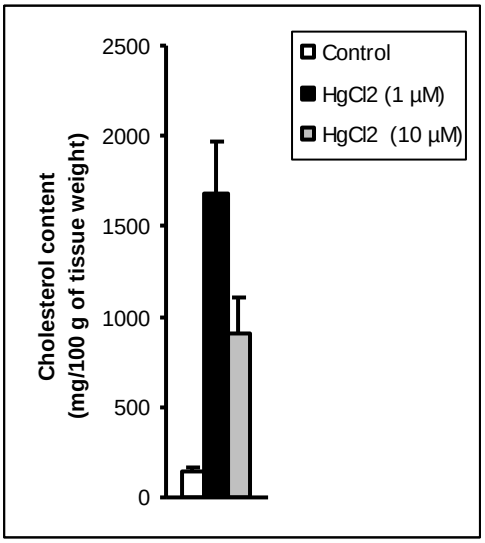


**Figure 01. Effects of $HgCl_2$ (1 and 10 µM) on cholesterol level in liver of *C. punctata*. The fish were treated with $HgCl_2$ (1 and 10 µM) for 1h. After the treatment, they were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving $HgCl_2$. The data are ± SE for 5 fish in each group.**

### Effects of mercuric chloride on triglyceride level in liver

Triglyceride turnover is a metabolic and biological process and is characteristic feature for the organisms so that they survive in the environment. The synthesis and degradation of triglyceride are essential cellular process and are influenced by alteration of the environmental stimulation. The toxic effects of mercury may impair the synthesis of triglyceride in liver. Therefore, to clarify whether $HgCl_2$ is involved in inducing triglyceride biosynthesis, groups of fish were exposed to different concentrations of $HgCl_2$ (1 and 10 µM) to examine the role of $HgCl_2$ on the changes of triglyceride in liver. As shown in Figure 02, the amount of triglyceride in liver of fish in response to $HgCl_2$ (1 µM) for 1h was 102.24 ± 16.12 mg while for 10 µM concentration, the value was found to 92.87 ± 14.47 mg/g



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of tissue weight. On the contrary, the amount of triglyceride in livers of group of fish (control) was recorded as 16.46 ± 3.07 mg/g of tissue weight. A significant increased (6.6 folds) ($p < 0.001$) response on triglyceride synthesis in liver was observed for fish exposed to $HgCl_2$ (1 µM). Similar stimulatory effects (5.6 folds) ($p < 0.001$) on triglyceride synthesis in liver were observed whenever fish were exposed to 10 µM concentrations; however the effects were assumed to be potential for the fish exposed to 1 µM concentrations of $HgCl_2$. The results indicated that mercury had been involved in impairment of triglyceride content in liver inducing lipogenesis and would suggest that this heavy element create an adverse environment and the increased triglyceride in liver may play the critical role to survive in this situation.

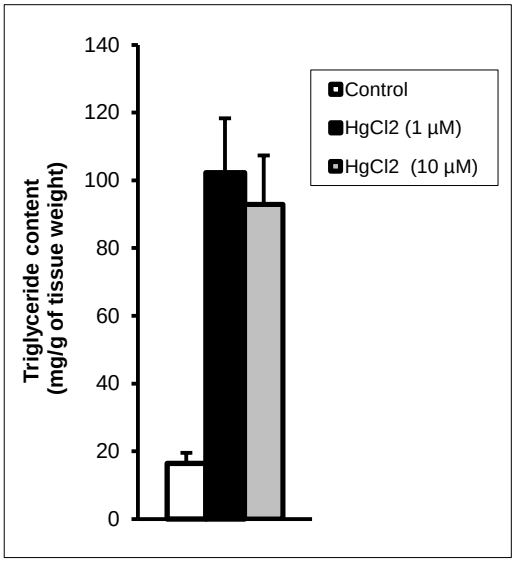


**Figure 02. Effects of $HgCl_2$ (1 and 10 µM) on triglyceride level in liver of *C. punctata*. The fish were treated with $HgCl_2$ (1 and 10 µM) for 1h. After the treatment, they were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving $HgCl_2$. The data are ± SE for 5 fish in each group.**

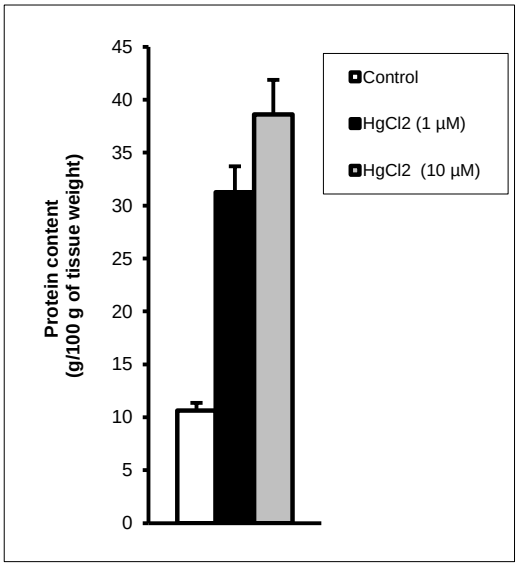


**Figure 03. Effects of $HgCl_2$ (1 and 10 µM) on protein level in liver of *C. punctata*. The fish were treated with $HgCl_2$ (1 and 10 µM) for 1h. After the treatment, they were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving $HgCl_2$. The data are ± SEM for 5 fish in each group.**



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**Effects of mercuric chloride on protein level in liver**

Mercury causes adverse and toxic environment through formation of oxidative stress and reactive oxygen species (ROS). However, to survive in such environment liver plays the critical role and generates stress proteins. Therefore, to examine whether mercuric chloride exposure is involved in the regulation of protein content in liver, groups of fish were treated with different concentrations (1 and 10 µM) of $HgCl_2$ for 1h. Control fish were used except giving $HgCl_2$. As shown in Figure 03, the average protein content in liver in response to $HgCl_2$ (1 µM) was 31.25 ± 2.47 g/100 g of tissue whereas for the control liver, the amount of protein was determined as 10.63 ± 0.72 g. A significant (2.9 folds, $p < 0.05$) increased protein level was observed after 1h when compared to the liver of control fish. Another group of fish were exposed to 10 µM concentration of $HgCl_2$ and the amount of protein was recorded as 38.62 ± 3.26 g/100 g of tissue after 1h. The results demonstrated that the protein content in liver had been enhanced significantly ($p < 0.05$) (3.6 folds) when they were exposed to 10 µM concentrations of mercuric chloride, compared to the control fish. The increased protein content was found to be higher for 10 µM than that of 1 µM concentration. The increased synthesis of protein in liver in response to toxic environment induced by mercury might be involved in the regulation of metabolic functions of this species of fish. The alteration of protein concentration in liver in response to mercury is an index for characterization of the sensitivity to the environmental stress. The increased protein in liver may involve in survival of the species of fish in the toxic environment created by mercuric chloride.

**Table 01. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation. Fish were treated with mercuric chloride (10 µM), calcium carbonate (1 mM) and mercuric chloride (10 µM) + $CaCO_3$ (1 mM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate**

| Treatments | Cholesterol content (mg/100 g of tissue weight) |
|---|---|
| Control | 145.73 ± 17.95 |
| $HgCl_2$ (10 µM) | 911.14 ± 194.75 [A] |
| $CaCO_3$ (1 mM) | 1459.79 ± 193.26 |
| $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) | 1523.01 ± 200.45 |

Data are means ± SE for 3~5 fish in each group. [A]$p < 0.001$ versus control for 1h.

**Role of calcium carbonate on cholesterol level in mercuric chloride induced liver**

As illustrated in Table 01, the effects of $CaCO_3$ (1 mM) on $HgCl_2$ (10 µM) induced liver of *C. punctata* were demonstrated. Groups of fish were treated with $HgCl_2$ (10 µM), $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) and $CaCO_3$ (1 mM). Cholesterol content in liver treated with $HgCl_2$ (10 µM) was 911.14 ± 194.75 mg while the value was recorded as 1523.01 ± 200.45 mg/100 g of tissue weight for fish treated with $HgCl_2$ and $CaCO_3$. The amount of cholesterol for fish treated with $CaCO_3$ (1 mM) was 1459.79 ± 193.26 mg/100 g of tissue weight and for the control, the value was recorded as 145.73 ± 17.95 mg/100 g of tissue weight. Mercuric chloride (10 µM) itself was found to cause the synthesis of cholesterol in liver when compared to the control, however, cholesterol content in liver was not reduced in response to 1 mM $CaCO_3$ rather increased when compared to the $HgCl_2$ treated liver non significantly. Figure 04 shows the effects of $CaCO_3$ (100 µM) on $HgCl_2$ (1 µM) induced liver of fish. Whenever, fish were exposed to $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM), cholesterol content was recorded as 476.98 ± 35.98 mg/100 g of liver while the value was found to 1678.42 ± 288.44 mg and 183.65 ± 73.67 mg for the fish treated with $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM) respectively. $CaCO_3$ (100 µM) causes a significant reduced cholesterol content by 71.5% ($p < 0.05$) compared to fish treated with $HgCl_2$ (1 µM) alone while 1 mM concentrations of $CaCO_3$ was failed to show such reducing effect on mercuric chloride induced liver. Mercuric chloride (1 µM) itself was involved in inducing cholesterol level. The results appeared to indicate that $CaCO_3$ might be involved in the prevention of the toxic effects of $HgCl_2$. in liver.



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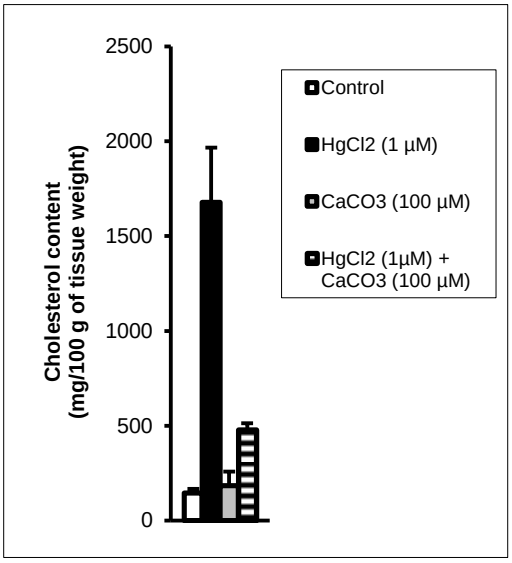


**Figure 04. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation of cholesterol. Fish were treated with mercuric chloride (1 µM), calcium carbonate (100 µM) and mercuric chloride (1 µM) + $CaCO_3$ (100 µM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate. Data are means ± SE for 3~5 fish in each group.**

**Table 02. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation. Fish were treated with mercuric chloride (10 µM), calcium carbonate (1 mM) and mercuric chloride (10 µM) + $CaCO_3$ (1 mM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate**

| Treatments | Triglyceride content (mg/g of tissue weight) |
|---|---|
| Control | 16.46 ± 3.07 |
| $HgCl_2$ (10 µM) | 92.87 ± 14.47 [A] |
| $CaCO_3$ (1 mM) | 71.57 ± 2.15 |
| $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) | 77.58 ± 12.75 |

Data are means ± SE for 3~5 fish in each group. [A]$p < 0.001$ versus control for 1h.

**Role of calcium carbonate on triglyceride level in mercuric chloride induced liver**

As shown in Table 02, the effects of $CaCO_3$ (1 mM) on $HgCl_2$ (10 µM) induced liver of *C. punctata* has been demonstrated. Groups of fish were treated with $HgCl_2$ (10 µM), $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) and $CaCO_3$ (1 mM). Control fish were used except giving $HgCl_2$ or $CaCO_3$ treatment. Triglyceride level in liver treated with $HgCl_2$ (10 µM) was 92.87 ± 14.47 mg while the value was recorded 77.58 ± 12.75 mg/g of tissue weight for fish treated with $HgCl_2$ and $CaCO_3$. The amount of triglyceride for fish treated with $CaCO_3$ (1 mM) was 71.57 ± 2.15 mg/g of tissue weight and for the control, the value was recorded as 16.46 ± 3.07 mg/g of tissue weight. Although the amount of triglyceride in response to $HgCl_2$ was enhanced significantly ($p < 0.001$) compared to control fish, triglyceride content in liver was reduced in response to 1 mM $CaCO_3$ by 16.4% when compared to the liver treated with $HgCl_2$ (10 µM) non-significantly. Whenever, fish exposed to $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM), triglyceride content was recorded as 19.87 ± 2.6 mg/g of liver weight while the value was found to 102.24 ± 16.12 mg and 27.14 ± 5.61 mg for the fish treated with $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM) respectively. It was found that $CaCO_3$ (100 µM) causes a significant reduced triglyceride content by 80.5% ($p < 0.01$) compared to $HgCl_2$ (1 µM) treated fish (Figure 05) and the effects were much pronounced than that of 1 mM concentration of $CaCO_3$ (Table 02, Figure 05). The results indicate that $CaCO_3$ is involved in the prevention of the toxic effects of $HgCl_2$ in liver and reduce triglyceride content.



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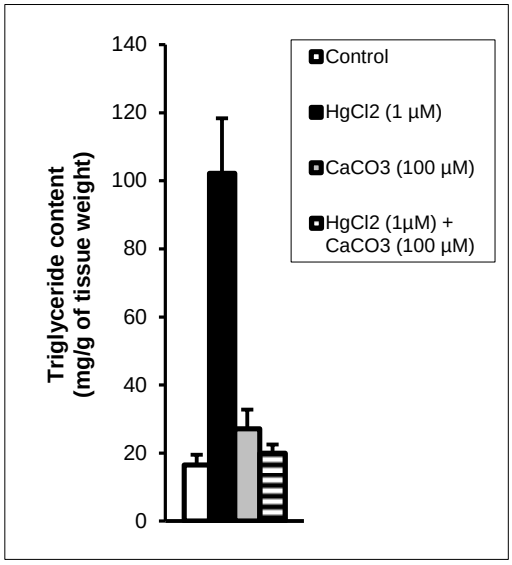


**Figure 05. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation of triglyceride. Fish were treated with mercuric chloride (1 µM), calcium carbonate (100 µM) and mercuric chloride (1 µM) + $CaCO_3$ (100 µM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate. Data are means ± SE for 3 fish in each group.**

**Table 03. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation. Fish were treated with mercuric chloride (10 µM), calcium carbonate (1 mM) and mercuric chloride (10 µM) + $CaCO_3$ (1 mM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate**

| Treatments | Protein content (g/100 g of tissue weight) |
|---|---|
| Control | 10.63 ± 0.72 |
| $HgCl_2$ (10 µM) | 38.62 ± 3.26 $^{A}$ |
| $CaCO_3$ (1 mM) | 21.02 ± 1.91 |
| $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) | 26.63 ± 2.29 $^{B}$ |

Data are means ± SE for 3~5 fish in each group. $^{A}p < 0.001$ and $^{B}p < 0.01$ versus control and $HgCl_2$ (10 µM) respectively for 1h.

**Role of calcium carbonate on protein level in mercuric chloride induced liver**

Both 1 and 10 µM concentrations of $CaCO_3$ also have been adopted to clarify the role of $CaCO_3$ on the toxic effects of $HgCl_2$ in liver of *C. punctata*. Table 03 shows the effects of $CaCO_3$ (1 mM) on protein content in $HgCl_2$ (10 µM) induced liver of *C. punctata*. Groups of fish were treated with $HgCl_2$ (10 µM), $HgCl_2$ (10 µM) + $CaCO_3$ (1 mM) and $CaCO_3$ (1 mM). The amount of protein in liver treated with $HgCl_2$ (10 µM) was 38.62 ± 3.26 g while the value was recorded as 26.63 ± 2.29 g/100 g of tissue weight for fish treated with $HgCl_2$ and $CaCO_3$. On the contrary, the amount of protein for fish treated with $CaCO_3$ (1 mM) was 21.02 ± 1.91 g/100 g of tissue weight and for the control, the value was recorded as 10.63 ± 0.72 g/100 g of tissue weight. The results demonstrate that the protein content in liver was increased significantly ($p < 0.05$) in response to $HgCl_2$ exposure, however the amount of protein content in liver was reduced in response to 1 mM $CaCO_3$ by 31.0% when compared to the control significantly ($p < 0.01$). Whenever, fish exposed to $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM), protein content was recorded as 12.06 ± 0.51 g/100 g of liver weight while the value was found to 31.25 ± 2.47 g and 18.35 ± 2.25 g for the fish treated with $HgCl_2$ (1 µM) and $CaCO_3$ (100 µM) respectively. $CaCO_3$ (100 µM) causes a significant reduced protein content in liver induced by $HgCl_2$ by 61.4% ($p < 0.01$) compared to fish treated with $HgCl_2$ (1 µM) and the effects were much potential than that of 1 mM concentration of $CaCO_3$ (Table 03, Figure 06). The results clearly demonstrate that $CaCO_3$ is involved in the prevention of the toxic effects of $HgCl_2$ in liver. The results appear to indicate that $CaCO_3$ might be involved in reducing the $HgCl_2$ induced protein synthesis in liver, however, both the chemical and environmental



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stresses seemed to cause the synthesis of stress proteins for the survival of the species of fish in that circumstances and the synthesis of protein is regulated in response to $CaCO_3$.

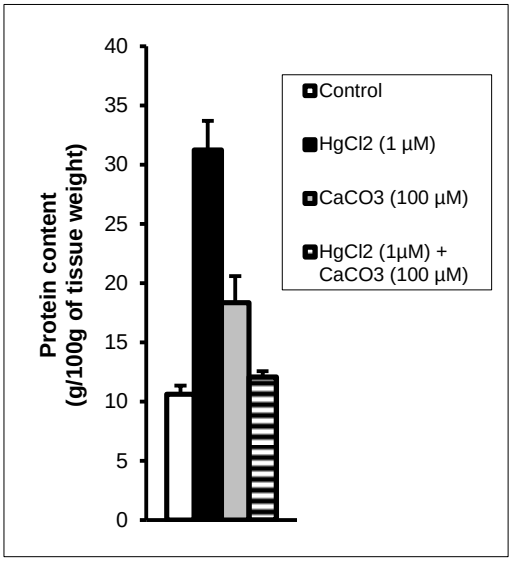


**Figure 06. Role of $CaCO_3$ on $HgCl_2$ induced metabolic regulation of protein. Fish were treated with mercuric chloride (1 µM), calcium carbonate (100 µM) and mercuric chloride (1 µM) + $CaCO_3$ (100 µM) for 1h. Control fish were similarly used except giving mercuric chloride or calcium carbonate. The data are means ± SE for 3 fish in each group.**

## IV. Discussion

Mercury (Hg) is an environmental toxic pollutant causing serious risks to the organisms and ecosystems (Chen *et al*., 2009). Inorganic Hg can be methylated by bacterial process to form a more toxic substance in aquatic ecosystems and is believed to have a more significant effect on aquatic animals (Zhang and Wong, 2007). Aquatic animals such as fish take up Hg either by direct exposure through their body or by ingestion. Hg can then bioaccumulate and biomagnify through the food chain (Alvarez *et al*., 2006). Uptake and elimination pathways differ substantially among tissues (e.g., liver, kidney, gills, and muscle), thus, Hg accumulation is tissue-specific (Rothschild and Duffy, 2005). The accumulation of Hg above certain levels in fish can result in serious biological disturbances or individual death. To date, a variety of adverse effects of Hg exposure have been observed in fish, including neurological, respiratory, immune, renal, dermatologic, reproductive, and developmental problems (Carta *et al*., 2003; Risher and Amler, 2005). The toxic effects of Hg are commonly associated with the formation of reactive oxygen species (ROS) in cells (Verlecar *et al*., 2008). The present article shows the role of $CaCO_3$ as a preventive measure for the toxic effects of mercury and was found to involve in reducing effectively the effects.

Different concentrations of $HgCl_2$ were used in this study where 1 µM rather than 10 µM concentrations were found to be involved predominantly in enhancing the metabolic activities particularly cholesterol, triglyceride and protein contents in liver. It has been demonstrated that Hg is involved in causing the toxic effects through formations of the reactive oxygen species (ROS) (Verlecar *et al*., 2007). These species might be involved in lipogenesis in liver through impairment of the lipid biosynthesis (Giudetti *et al*., 2013). Triglyceride turnover is an essential biological process of the organisms. Triglyceride degradation causes fatty acids and glycerol and is oxidized to get energy. The increased triglyceride in response to $HgCl_2$ is also a metabolic process and might be involved in survival of the species in adverse environment. Although, $HgCl_2$ is toxic to the living organisms, fish survive in water for 1h in response to 1 or 10 µM concentration of $HgCl_2$. Moreover, liver is a major tissue where biotransformation of foreign toxic substances has been observed. It is probable that increased synthesis of triglyceride in response to $HgCl_2$ is a metabolic process although detoxification of $HgCl_2$ might be involved in maintaining the homeostasis of triglyceride in liver tissue. Although



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much evidence were not observed in response to $HgCl_2$ on the enhancement of triglyceride, the previous study reveals that arsenic, a potent toxic and heavy element similar to Hg causes the similar effects and produces the fatty liver with increasing liver weight (Roy and Haque, 2009). Ung *et al.* (2010) found that lipid accumulation as indicated by the increased number and size of red-stained lipid vesicles, was identified in the liver of $HgCl_2$-treated fish. The transcriptome analysis shows clearly that up-regulation of fatty acid synthesis and down-regulation of mitochondrial fatty acid β-oxidation were found in the liver of $HgCl_2$-treated zebrafish. Deposition of lipids thus leads to adipogenesis or fatty liver diseases. It was found that the transcription factors CCAAT/enhancer-binding proteins (C/ebps) are believed to induce gene expression and cause adipogenesis (Rangwala and Lazar, 2000; Rosen *et al*., 2000). Therefore, the results are good agreement with their findings. Liver is the major organ playing the critical role in metabolic regulations and is involved in survival of the species as liver glycogenolysis is one of the important biological processes causing the energy output. During adverse environment caused by mercury treatment, the species wants to survive although the mechanism is not well clarified however, recent investigations reveals that formation of stress proteins participates in the survival process (Al-Whaibi, 2011). Cholesterol is another molecule available in liver however the amount of cholesterol in response to $HgCl_2$ was increased showing the higher lipogenesis in liver. The increased synthesis of cholesterol also may induce higher liver weight and fatty liver as demonstrated by Roy and Haque (2009). In the current study, mercuric chloride was involved in enhancing protein content in liver. Therefore, the increased protein in response to Hg might be involved in this connection. Fish are considered as suitable biomonitors for environmental pollution and they are exposed to the heavy metals in vitro and to study the effects of heavy metals in aquatic ecosystems (Padmini *et al*., 2004). Up- regulation of heat shock protein genes, oxidative stress-inducible genes and genes coding for proteins associated with antioxidant activity suggests increased oxidative stress and reactive oxygen species (ROS) in liver of mercury treated fish (Li *et al*., 2014).

Biochemical mechanisms are involved in detoxification of foreign substances particularly the deleterious effects of various metals or other environmental toxicants (Lopez *et al*., 2001) and exposure of biomarkers to aquatic pollutants (Bainy, 1996; Ahmad *et al*., 2006). Although the mechanism of $CaCO_3$ action is not clarified however, it is assumed that $Ca^{2+}$ might be involved in the following ways: i) it may reduce the oxidative stress caused by $HgCl_2$ in the liver cells. The increased cholesterol, triglyceride or protein might be due to the higher oxidative effects as this element (Hg) is believed to be involved in causing the formation of reactive oxygen species (Verlecar *et al*., 2007). Therefore, the increased biosynthesis of these molecules in response to $HgCl_2$ is prevented by the treatment of $CaCO_3$ and ii) whenever the cells were treated with $HgCl_2$, the oxidative process particularly TCA might be impaired thereby during glycolysis process, acetyl-CoA produced is not further converted through TCA rather is used to form cholesterol or triglyceride. Calcium ion might be involved in impairing this step and inhibit the formation of these molecules. Different concentrations of $CaCO_3$ were used however, 100 µM rather than 1 mM concentration effectively reduced the effect of mercury showing the higher efficiency of reducing the effect at lower dose of $CaCO_3$. Various environmental factors affect the behavior of metals in aquatic ecosystems. As demonstrated by Das and Datta (2008) that the toxic effect of mercury has been shown to be reduced by contact time with soil sediment. Higher the time of contact, greater the effect was observed. Datta *et al*. (2003) reported the complexation of mercury with inorganic and organic ligands of water solutions (e.g. carbonate dissolved organic carbon and bicarbonates) and adsorption of Hg in particulate organic carbon (POC), clay and iron or manganese oxyhydroxides. Their findings suggest that the toxic effects of Hg might be prevented by $CaCO_3$ in solution. In their experiment, higher toxicity was seen in lower sediment chemical contact time and vice versa. This was due to the complexity and adsorption of inorganic mercury ions with the dissolved solutions of water and soil sediment respectively reduced when the contact time was less and therefore, accumulation of mercury by aquatic fish were more. This was also supported by the observation of Jackman *et al*. (2001) who observed that time necessary for the absorption equilibrium increases with the increasing concentration of polluted solutions. The interparticle migration of metal cations has been found to proceed at significant rates that were affected by the length of the time at which the metal was in contact with the soil sediment. Collectively, heavy metals like Hg is toxic and affects the metabolic activities particularly lipid or protein metabolism and $CaCO_3$ does play the effective role in prevention of the metabolic and deleterious effects of $HgCl_2$.



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## V. Conclusion

As a heavy element, Hg was found to be involved in impairment of cholesterol, triglyceride and protein in liver. The increase in these molecules might be due to the oxidative stress caused by mercury since this heavy element is well known to be involved in causing higher oxidative stress in the environment. Therefore, it is substantial to prevent this oxidative stress making the suitable and normal environment where these species of fish will survive. Although the exact mechanism of this complex phenomenon is not clarified, however in the current research, the role of $CaCO_3$ has been adopted and found to be involved in prevention of the toxic effects of mercury. The metabolite regulation in response to Hg exposure might be involved in survival of these species and represents a substantial biochemical process. As a peripheral tissue, liver plays the dominant role in metabolic regulation and involved in detoxification of foreign toxic compounds. This peripheral tissue has been recognized to be a metabolically significant for energy consumption and energy release however the adverse metabolic functions (cholesterol, triglyceride and protein biosynthesis) induced by $HgCl_2$ is effectively and appreciably prevented by $CaCO_3$ in aqueous system.

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