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METABOLIC ALTERATIONS IN LIVER OF FRESH WATER FISH, *C. PUNCTATA* EXPOSED TO ARSENIC: AN ADVERSE AND ADAPTIVE RESPONSE TO THE ENVIRONMENT

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Abstract

Arsenic is a major toxicant impairing in diverse metabolic alterations of the organisms and the mechanism of adaptive response is yet to be identified. In the present study, effects of different doses of arsenic in liver of *Channa punctata* on the regulation of metabolic activities were done. *C. punctata*, a variety of fresh water fish were exposed to 1, 10 and 1000 μM concentration of Na_2HAsO_4 for 1 h. The amount of protein, in response to 1, 10 and 1000 μM concentration of arsenic were augmented by 184.47% (2.84-folds), 202.82% (3.0-folds) and 317.49% (4.17-folds), respectively and was found to be higher for 1000 μM dose. Cholesterol contents in liver were similarly exaggerated by 517.45% (6.17-folds), 308.13% (4.1-folds) and 286.41% (3.86-folds), respectively. However, the higher response was found for 1 μM dose of Na_2HAsO_4 . Similar stimulatory effects on triglyceride level were observed in response to arsenic. Na_2HAsO_4 causes 443.74% (5.43-folds), 533.11% (6.33-folds) and 548.48% (6.48-folds) enhanced triglyceride level in liver respectively and the effects were pronounced for 1000 μM concentration. Our findings conclude that arsenic is involved in impairment of metabolic activities in liver of the species of fish and gives an impact to the environment for survival.

Keywords: *Channa*, Liver, Metabolic Regulation, Arsenic, Oxidative Effects

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Introduction

Arsenic is a major toxicant and causes severe oxidative stress to the environment (Samuel *et al.*, 2005; Muthumani and Miltonprabu, 2012). Numerous epidemiological studies have demonstrated that exposure to arsenic is associated with an increased incidence of lung, bladder, and skin cancer, as well as diabetes and cardiovascular disease (Erraguntla *et al.*, 2012). Arsenic is an element that is present at low concentrations everywhere such as in air, soil and water (Gupta *et al.*, 2005). Compounds of arsenic, concentrated in the environment, as a result of natural or anthropogenic sources, become a major concern for environmental and occupational health (Gupta *et al.*, 2005; Rana *et al.*, 2010). Eventually, oxidative stress may occur partially with arsenic toxicity (Kalia *et al.*, 2007; Banerjee *et al.*, 2009). Therefore, it is well recognized that the accumulation of arsenic to the living organism affects metabolic impairment and

has been adversely affected to the tissues of aquatic organisms. Among the tissues, liver plays the critical role in metabolic regulation particularly carbohydrate, protein and lipid metabolism. Therefore, arsenic induced metabolic alteration in liver is an important aspect in the current study. It has been demonstrated that exposure of arsenic to aquatic fish causes the formation of oxidative stress (Mahboob, 2013). Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences in living organisms (Nishida, 2011). Their findings suggest that antioxidative effects caused by heavy elements might be the synthesis of some essential molecules substantial for the survival of the aquatic fish although not clarified well.

C. punctata (Taki fish) has been well characterized and is generally found in fresh water of haor, beel and river of Bangladesh. They are much energetic and survive in the critical circumstances for long time. They are the major sources of protein in the diet for human being. It is assumed that the higher energy content of this fish is caused by the increased activity of the sympathetic nerves. Peripheral tissue metabolism is affected by both environmental and chemical stimuli; however, endogenous auto regulation of metabolic processes of all species is a common biological process. To survive in adverse environment, this biological process plays the vital role either the formation of some compounds or degradation of the molecules. Arsenic causes the polluted environment; when the concentration of the pollutants exceeds the normal range, the organisms wants to survive. Therefore, arsenic induced intoxication is an important issue and the regulation of metabolic functions is an important aspect to clarify the mechanism of arsenic toxicity. Studies (Ghatak *et al.*, 2011) on chronic arsenic toxicity have revealed that oxidative stress and hepatic stellate cell activation are key events in arsenic induced liver fibrosis. Increasing the antioxidant capacity of cells thereby has become a therapeutic strategy to antagonize arsenic poisoning (Rana *et al.*, 2010). Degradation of biomolecules as well as biosynthesis is the characteristics of metabolic processes under some special circumstances. Among the peripheral tissues, the skeletal muscle and the liver play a great role in metabolic regulation. The metabolic functions in these tissues are influenced by both environmental and chemical stimuli. Liver glycogenolysis is a metabolic process yielding energy for doing mechanical work and the process is enhanced upon activation of the sympathetic nervous system. Although, *C. punctata* has been localized to the environment where the deficiency of water and other adversity have been observed however the survival of these fish to the environment is sometimes impaired by the environmental pollutants particularly when the concentration of the compounds exceeds the normal range. In this circumstance, the alteration of metabolites may enhance the survival to the environment. Therefore, it is necessary to find strategy for the prevention of the toxic effects of arsenic (As) which causes environmental pollution and impairment of metabolic regulation in fish and other aquatic organisms. Although heavy elements like arsenic cause severe oxidative stress and environmental pollution, however survival to the toxic environment is an important aspect for the aquatic organisms and its mechanism is not well characterized and how arsenic is involved in impairment of metabolic activities in liver of this species is not clarified. Therefore, the current study has been undertaken regarding the acute

effects of arsenic compounds on the regulation of metabolic activities in liver of fresh water fish *C. punctata*.

Materials and Methods

Fish

Channa punctatus weighing 50 g to 60 g were used and maintained in normal water with ambient temperature (25.0 ± 1 °C). In the day of experiment, exposure of arsenic (Na_2HAsO_4) was given to the different groups of fish in small plastic pots for 1 h period with full aeration and with free access of water. After the treatment, fish were quickly decapitated and liver was sampled carefully and taken weight by digital balance (Chyo, JL-180, China) and kept at -20°C . Control fish were similarly used for sampling of tissue except giving arsenic exposure.

Arsenic treatment

To examine the role of arsenic on the regulation of metabolic activity involving the amount of protein, triglyceride and cholesterol in liver of *C. punctatus*, fish were exposed to sodium arsenate (Na_2HAsO_4 , BDH Chemical Ltd.) in water (500 mL) for 1 h. The groups of fish were treated with different concentration of sodium arsenate (1, 10 and 1000 μM Na_2HAsO_4) in water (500 mL) while control fish were exposed to similar volume of water for 1 h only. The tissue was sampled after the treatment similarly as mentioned above and analyzed for different metabolites.

Assay of tissue protein content

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 0.1 N NaOH) and 1.0 mL of copper-sodium potassium tartarate solution (1 g sodium potassium tartarate and 0.5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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 g 100 g^{-1} of tissue weight.

Assay of tissue triglyceride content

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 μ L of crude liver sample were used.

Assay of tissue cholesterol 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-H₂SO₄ mixture (20 mL of acetic anhydride and 1 mL of concentrated H₂SO₄) 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⁻¹ 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-H₂SO₄ mixture were used. The amount of cholesterol was expressed as mg 100 g⁻¹ 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.

Results

Effects of Na₂HAsO₄ on protein level in liver of *C. punctata*

Arsenic causes the formation of oxidative stress and reactive oxygen species (ROS) and thereby making the adverse and toxic environment. However, to survive in such environment liver plays the pivotal role and generates stress proteins. To examine whether sodium arsenate (Na₂HAsO₄) exposure is involved in the regulation of protein content in liver, groups of fish were treated with different concentrations (1 and 10 and 1000 μ M) of Na₂HAsO₄ for 1 h. Control fish were used except giving Na₂HAsO₄.

Table 1. Effects of arsenic on protein level in liver of *C. punctata*. The groups of fish were treated with Na₂HAsO₄ (1, 10 and 1000 μ M) for 1 h. After the treatment, the fish were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving Na₂HAsO₄.

Treatments	Protein content (g 100 g ⁻¹ of tissue weight)
Control	10.63 \pm 0.72
Arsenic (1 μ M)	30.24 \pm 2.96 ^A
Arsenic (10 μ M)	32.19 \pm 2.34 ^B
Arsenic (1000 μ M)	44.38 \pm 7.06 ^B

The data are means \pm SE for 5 fish in each group. ^A*p* < 0.05 and ^B*p* < 0.05 versus control for 1 h.

As shown in Table 1, the average protein content in liver in response to Na₂HAsO₄ (1 μ M) was 30.24 \pm 2.96 g 100 g⁻¹ of tissue whereas for the control liver, the amount of protein was determined as 10.63 \pm 0.72 g. A significant (184.47%; 2.84-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 μ M concentration of Na₂HAsO₄ and the amount of protein was recorded as 32.19 \pm 2.34 g 100 g⁻¹ of tissue after 1h. The results demonstrated that the protein content in liver had been enhanced significantly (*p* < 0.05) (202.82%; 3.0-folds) when they were exposed to 10 μ M concentrations of sodium arsenate, compared to the control fish. The increased protein content was found to be higher for 10 μ M than that of 1 μ M concentration (Fig. 1). Similar

stimulatory effects were also observed whenever fish were exposed to higher concentrations (1000 μ M) of Na₂HAsO₄ where Na₂HAsO₄ causes the formation of protein contents, 44.38 \pm 7.06 g 100 g⁻¹ of tissue. A significant (317.49%; 4.17-folds, *p* < 0.05) increased protein level was observed after 1h when compared to the liver of control fish (Table 1, Fig. 1). The increased synthesis of protein in liver in presence of toxic environment induced by arsenic (As) might be involved in the regulation of metabolic functions of this species of fish. The alteration of protein concentration in liver in response to arsenic is an index for characterization of the sensitivity to the environmental stress. The increased protein in liver may contribute to survival of the species of fish in the toxic environment created by Na₂HAsO₄.

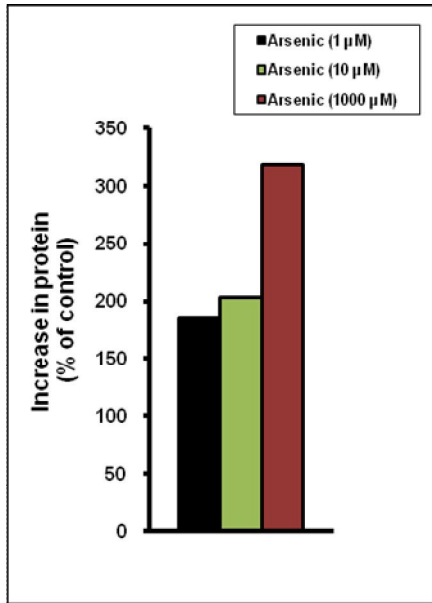


Fig. 1. Increase in metabolite (protein) in liver of *C. punctata* in response to different concentrations of Na₂HAsO₄. The results are expressed as the percentage of control.

Effects of Na₂HAsO₄ on cholesterol level in liver of C. punctata

Sodium arsenate (Na₂HAsO₄) is toxic to the living organisms. Exposure of higher concentration of arsenic 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 arsenic in liver is an important aspect in liver metabolism. The cellular uptake of arsenic may impair lipid metabolism. To clarify whether Na₂HAsO₄ affects cholesterol level in liver, groups of fish (*C. punctata*) were exposed to Na₂HAsO₄ (1 and 10 and 1000 µM). After the treatment, liver was excised and cholesterol content in liver was determined. Control fish were similarly used except Na₂HAsO₄ treatment. As shown in Table 2, the average cholesterol content in liver of fish exposed to 1 µM concentration of Na₂HAsO₄ was 899.82 ± 112.65 mg while for the control fish, the value was 145.73 ± 17.95 mg 100 g⁻¹ of tissue weight.

Table 2. Effects of arsenic on cholesterol level in liver of *C. punctata*. The groups of fish were treated with Na₂HAsO₄ (1, 10 and 1000 µM) for 1 h. After the treatment, the fish were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving Na₂HAsO₄.

Treatments	Cholesterol content (mg 100 g ⁻¹ of tissue weight)
Control	145.73 ± 17.95
Arsenic (1 µM)	899.82 ± 112.65 ^A
Arsenic (10 µM)	594.78 ± 51.99 ^B
Arsenic (1000 µM)	563.12 ± 74.10 ^B

The data are means ± SE for 5 fish in each group. ^A*p* < 0.001 and ^B*p* < 0.001 versus control for 1 h.

The results show that cholesterol content in liver was increased significantly (517.45%; 6.17-folds) (*p* < 0.001) when compared to the liver of control fish. Groups of fish were exposed to arsenic with 10 µM concentrations of Na₂HAsO₄ solution and the cholesterol content in liver was recorded as 594.78 ± 51.99 mg 100 g⁻¹ of tissue. The cholesterol level in response to Na₂HAsO₄ was found similarly to be increased when compared to control (308.13%; 4.1-folds; *p* < 0.001) however the results were found to be lower than that of 1 µM of Na₂HAsO₄. The amount of cholesterol was recorded as 563.12 ± 74.10 mg 100 g⁻¹ of tissue whenever fish were exposed to Na₂HAsO₄ (1000 µM). The cholesterol content was similarly and significantly increased (286.41%; 3.86-folds; *p* < 0.001) in response to higher dose of arsenic compound when compared to control fish (Fig. 2, Table 2) and the results were much lower than that of 1 µM of Na₂HAsO₄ (Table 2).

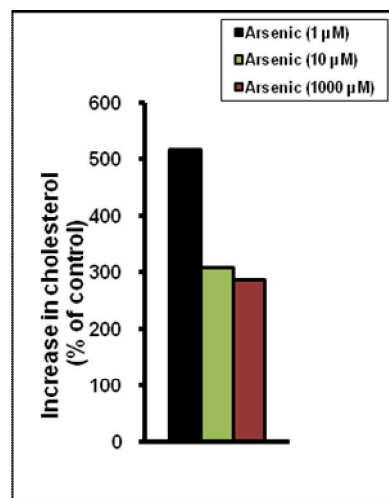


Fig. 2. Increase in metabolite (cholesterol) in liver of *C. punctata* in response to different concentrations of Na₂HAsO₄. The results are expressed as the percentage of control.

The results would suggest that arsenic causes the toxic environment and oxidative stress might be induced where the species of fish survive, thereby stimulates the synthesis of cholesterol in liver. The higher cholesterol level in liver may play the critical role in survival of the species in such environment. Therefore, it is assumed that both chemical and environmental factors cause the adverse effects and are involved in enhancing the accumulation of cholesterol in liver.

Effects of Na₂HAsO₄ on triglyceride level in liver of C. punctata

Triglyceride turnover is a metabolic and biological process and represents a characteristic feature for the organisms so that they can survive in the environment. The synthesis and degradation of triglyceride are essential biochemical process and are influenced by alteration of the environmental stimulation. The toxic effects of arsenic may impair the synthesis of triglyceride in liver. Therefore, to clarify whether Na₂HAsO₄ is involved in inducing triglyceride biosynthesis, groups of fish were exposed to different concentrations of Na₂HAsO₄ (1 and 10 and 1000 µM) to examine the role of arsenic on the changes of triglyceride in liver. As

shown in Table 3, the amount of triglyceride in liver of fish in response to Na₂HAsO₄ (1 µM) for 1h was 89.50 ± 8.04 mg while for 10 µM concentration, the value was found to 104.21 ± 13.51 mg g⁻¹ 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⁻¹ of tissue weight. A significant increased (443.74%; 5.43-folds; *p* < 0.001) response on triglyceride synthesis in liver was observed for fish exposed to Na₂HAsO₄ (1 µM). Similar stimulatory effects (533.11%; 6.33-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 10 µM concentrations of Na₂HAsO₄ rather than that of 1 µM dose (Fig. 3). To find the effects of higher concentrations of arsenic, fish were exposed to 1000 µM concentration of sodium arsenate. Arsenic causes the formation of higher triglyceride contents (106.74 ± 20.89 mg g⁻¹ of tissue weight) in liver after 1h and the results are increased by 6.48-folds (548.48%; *p* < 0.001) when compared to control fish (Table 3, Fig. 3).

Table 3. Effects of arsenic on triglyceride level in liver of *C. punctata*. The groups of fish were treated with Na₂HAsO₄ (1, 10 and 1000 µM) for 1 h. After the treatment, the fish were immediately decapitated and sampling of tissue was performed. Control fish were similarly used except giving Na₂HAsO₄.

Treatments	Triglyceride content (mg g ⁻¹ of tissue weight)
Control	16.46 ± 3.07
Arsenic (1 µM)	89.50 ± 8.04 ^A
Arsenic (10 µM)	104.21 ± 13.51 ^B
Arsenic (1000 µM)	106.74 ± 20.89 ^B

The data are means ± SE for 5 fish in each group. ^A*p* < 0.001 and ^B*p* < 0.001 versus control for 1 h.

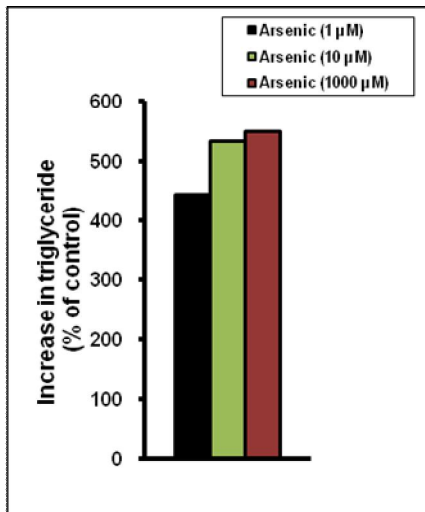


Fig. 3. Increase in metabolite (triglyceride) in liver of *C. punctata* in response to different concentrations of Na₂HAsO₄. The results are expressed as the percentage of control.

The results demonstrated that arsenic had been involved in impairment of triglyceride in liver inducing lipogenesis and would suggest that this heavy element creates an adverse environment and the increased triglyceride in liver may play the critical role to survive in this circumstance.

Discussion

The metabolic regulation in liver of *C. punctata* in response to sodium arsenate has been focused in the current investigation. Sodium arsenate (Na₂HAsO₄) is an arsenic species involved in severe impairment of metabolic processes. The tissue uptake and its severe deleterious effects have been reported in several investigations (Rahman *et al.*, 2009; Ei-Demerdash *et al.*, 2009). Compounds of arsenic concentrated in the environment causing environmental pollution therefore considered to be the major concern for environmental and occupational health. It has been demonstrated that exposure of arsenic to living organisms causes severe oxidative stress (Muthumani and Miltonprabu, 2012) thereby the

cellular metabolic impairment and tissue damage have been observed. It is therefore the diverse investigations of arsenic are needed and its preventive measure is substantial. In the current investigations, sodium arsenate has been found to be involved in the regulation of metabolism of protein, triglyceride and cholesterol in liver of *C. punctata*. Short term (1 h) exposure of arsenic causes severe stimulatory effects on protein, triglyceride and cholesterol content. Although, the mechanism is not clear that how this heavy element causes the higher synthesis of these metabolites, it is assumed that this element might be involved in the higher formation of reactive oxygen species (ROS) and for the adaptive response of the species of fish, these molecules play the critical roles in survival of the species since arsenic has been shown to be involved in causing ROS (Muthumani and Miltonprabu, 2012).

Exposure of arsenic has been shown to trigger protein in liver of *C. punctata*. To survive in the toxic environment, the organisms may cause the formation of some stress proteins which may play the role in this respect. Arsenicals are also known to induce a number of major stress protein families, including heat shock proteins (hsp) both *in vitro* and *in vivo* in several organs and systems with a rapid dose dependent response to acute exposure to arsenite (Gornati *et al.*, 2002). It has been found that arsenic induced liver of aquatic fish shows higher expression of proteins (stress proteins) (Roy and Bhattacharya, 2006) since this protein may act in the defense system. Fish are considered as suitable biomonitors for environmental pollution and they are exposed to the heavy metals *in vitro* to study the effects of heavy metals in aquatic ecosystems (Padmini *et al.*, 2004). The results are agreement with the present investigation showing the increased protein in liver of *C. punctata* treated with Na_2HAsO_4 . Fish treated with Na_2HAsO_4 (1, 10 and 1000 μM) shows higher protein contents in liver when compared to control fish. Both chemical and environmental stimuli cause higher oxidative effects therefore, it is reasonable and rational that the protein contents synthesized in liver were found to be higher. The previous study reveals that cold sensitive stress proteins were synthesized in liver of individuals in response to environmental stimuli (Ibarz *et al.*, 2010). Their findings are compatible and correlated with the present investigations.

Cholesterol is another molecule available in liver however the amount of cholesterol in response to Na_2HAsO_4 was increased showing the higher lipogenesis in liver. The increased synthesis of cholesterol also may induce higher liver weight and fatty liver and cause lipogenesis as demonstrated by Kumar and Banerjee (2012). In separate experiments, groups of fish were

exposed to Na_2HAsO_4 for 1 h and cholesterol contents in liver were recorded. The enhanced cholesterol levels were observed in response to different doses of Na_2HAsO_4 when compared to control fish exposed to water only. The increased cholesterol might be due to the higher oxidative effects as these stimuli have been believed to be involved in augmentation of causing of oxidative stress. Moreover, it has been shown that arsenic exposure to fish potentially stimulates the liver cells and increases the total liver mass and lipid content (Johnston and Dunn, 1987; Kent *et al.*, 1988). Cholesterol is a sterol compound synthesized in liver and is used to produce hormones and cell membranes. It is transported in the blood plasma of all mammals and is required to establish proper membrane permeability and fluidity. Therefore, synthesis of cholesterol in liver may contribute to other functions of the organisms.

Although much evidence were not observed in presence of Na_2HAsO_4 on the enhancement of triglyceride, the previous study reveals that arsenic, a potent toxic and heavy element causes the similar effects regarding the synthesis of lipids in liver of fresh water fish (Kumar and Banerjee, 2012). The lipid accumulation, as indicated by the increased number and size of red-stained lipid droplets, was detected in the liver of arsenic-treated fish (Li *et al.*, 2016). The histological phenotype was thus consistent with the transcriptome analysis where up-regulation of fatty acid synthesis and down-regulation of mitochondrial fatty acid β -oxidation might be observed in the liver of arsenic-treated zebrafish. It is possible that upon arsenic treatment, fat accumulates in the liver as lipid esters in droplets while free fatty acids reduce or remain unchanged. Cellular fatty acid composition is affected and lipid droplets dramatically increased, which could be a result of triglyceride built up and compromised lipoprotein metabolism. Accumulation of lipids can lead to adipogenesis, steatostasis or non-alcoholic fatty liver diseases. The results of the present investigation therefore, are good agreement with their findings. Triglyceride contents in liver were affected in presence of Na_2HAsO_4 and demonstrated to be enhanced when compared to the effects of control fish. The lipogenesis is a metabolic process in liver and has been demonstrated to be influenced by the environmental stimulus (Das *et al.*, 2013). Their findings indicate that environmental stress causes the enhancement of gluconeogenesis which might be linked to lipogenesis in liver. The augmented triglyceride in liver of *C. punctata* in the current investigation when exposed to environmental stress might be also because of the higher oxidative effects. The previous investigations reveal that exposure to heavy elements like arsenic influences fish liver, enhances lipid

content and total liver mass (Johnston and Dunn, 1987; Kent *et al.*, 1988). It is therefore, assumed from the above evidence that the increased triglyceride in presence of arsenic is because of the increased lipogenesis process.

Although arsenic induced, augmentation of diverse metabolites in liver has been found in the current study however, the mechanism is not clarified well. Several lines of evidences may support in the arsenic induced mechanism. Antioxidant response is the major cellular defense mechanism that a cell has against arsenic insult. Arsenic-induced antioxidant imbalance has been reported in numerous studies (Valko *et al.*, 2005; Samuel *et al.*, 2005). These studies have demonstrated that various enzymatic and nonenzymatic factors help in protecting cells by scavenging and clearing ROS. The ability of glutathione, the most abundant nonprotein thiol in cells, to react with electrophiles directly or as a cofactor for enzymes (GPx and GST) may play an important role in arsenic detoxification and against arsenic-induced oxidative stress. Mitochondria are the prime targets for arsenic intoxication, either indirectly via ROS accumulation or directly through condensing mitochondrial matrix and opening of permeability transition pores by virtue of its thiol-oxidizing property. In either case, arsenic-induced mitochondrial insult initiates apoptosis (Pulido and Parrish, 2003). Arsenic intensively affects the ROS metabolizing enzymes called antioxidant enzymes, such as SOD, CAT, glutathione peroxidase (GPx), GST, and glutathione reductase (GR). Generally, short-term exposure to low arsenic concentrations results in an increase in the activity of these enzymes, whereas chronic exposure usually results in their reduction. Arsenic is also known to regulate the activity of thioredoxin reductase, heme oxygenase reductase, and NADPH oxidase *in vitro* (Kitchin and Conolly, 2010) and it is also known to alter activities of cytochrome P450s. Arsenic is also shown to inhibit pyruvate dehydrogenase (PDH) activity either via oxidative damage or through binding to vicinal dithiols in both pure enzyme and tissue extract. However, its concentration required to deactivate the enzyme is much lower than what is required for direct binding to thiol groups, suggesting an alternative mechanism (Samikkannu *et al.*, 2003).

Although diverse investigations reveal the concept of the mode of arsenic in the biological system, the detoxification of this element is substantial and the strategy finding the prevention of arsenic toxicity is more important in this respect. Prevention of heavy metal toxicity may be accomplished by either reducing the possibility of metal interacting with critical biomolecules and inducing oxidative damage or

by increasing the cells antioxidant defenses through endogenous and exogenous supplementation of antioxidant molecules (Kalia *et al.*, 2007). To minimize toxic effects and damages caused by arsenic, cells have improved defense systems, which include antioxidant molecules. When toxic agents against the natural protective systems overrun, exogenous antioxidative and protective compounds must be taken (Devillers *et al.*, 2001). The current investigations reveal that Na_2HAsO_4 is a major adaptive and oxidative stress and have been shown to be involved in regulation of the biochemical processes in liver of *C. punctata*. The regulation of different metabolites in liver of the species may enhance the identification of arsenic induced causes and give the clue to the concept of prevention.

Conclusion

In the current investigation, diverse metabolic alterations in liver of *C. punctata* in response to arsenic have been noticed. Arsenic is a major toxicant causing environmental pollution and has been found to be involved in liver metabolic functions. Different doses of arsenic have been severely found to be involved in stimulation of the metabolic activities. Arsenic accumulation in liver affects protein, cholesterol and lipid metabolism. Although the mechanism is not clarified, however it is assumed that the impairment caused by arsenic might be because of the higher oxidative stress. In severe toxic and adverse environment, these species of fish wants to survive. Therefore, current investigation may augment and give a new insight on the adaptive response to the adverse environment for these species of fish.

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