CHAPTER IV

BIOCHEMICAL PARAMETERS

INTRODUCTION

Exposure of fish to xenobiotics leads to interactions between the chemicals and biological systems, which give rise to biochemical disturbances (Monteiro et al., 2006). Prominently, the biochemical alterations are usually the first detectable and quantifiable responses to environmental changes of animal health and internal environment of the organism (Agrahari et al., 2007; Kavitha et al., 2010; Li et al., 2011; Saravanan et al., 2011a). Agrahari et al. (2007) reported that analysis of biochemical parameters could help to identify target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism. Biochemical parameters were often used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances (Osman et al., 2010). In general, biochemical profiles in fish and other aquatic organisms under stress serve as important bioindicators in the monitoring of aquatic environment.

Fish blood is widely used in toxicological research and environmental monitoring as a promising indicator of physiological and pathological changes of the whole body (Mahttiessen et al., 1995; Velisek et al., 2010). Blood plasma reflects the physiologic state of an animal because they are the products of intermediate metabolism (Artacho et al., 2007; Firat and Kargin, 2010) and the parameters of blood plasma are important in diagnosing the structural and functional status of fish (Adhikari et al., 2004). Measurement of plasma biochemical parameters is mostly used in clinical diagnosis of fish physiology to determine the general status of health (Ferreira et al., 2007; Osman et al., 2010). Hence, the biochemical parameters of blood in aquatic organisms are used as possible biomarkers to evaluate the toxicity of various chemicals (Tellez-Banuelos et al., 2009; Saravanan et al., 2011; Banaee et al., 2011). Recently, these biomarkers are widely employed to assess the impacts of pharmaceuticals on fish and other aquatic organisms to attain early-warning signals of environmental risks (Fent et al., 2006; Ballesteros et al., 2009; Santos et al., 2010; Quinn et al., 2011).
Among the biochemical parameters, plasma glucose and protein are extensively used to assess the impacts/stress induced by environmental contaminants (El-Sayed et al., 2007; Remyla et al., 2008; Lavanya et al., 2011, Saravanan et al., 2011a). It is well known that carbohydrate and protein play a vital role as energy precursors for fish under stress conditions (Umminger, 1970; Sornaraj et al., 2007). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Soengas and Aldegunde, 2002; Martínez-Porchas et al., 2009). Generally, stress response includes increases in plasma glucose and lactate (Hontela et al., 1996; Santos and Pacheco, 1996) level. It has been reported that the increased blood glucose is usually observed in fish under undesirable conditions and it helps the animal by providing energy substrates to vital organs to cope with the increased energy demand (Velisek et al., 2006; Banaee et al., 2008; Saha and Kaviraj, 2009). According to Chavin (1973) blood glucose and hepatic glycogen can be utilized as a parameter of stress response, as it is rapid, practicable and quantitative (Hattingh, 1976; Donaldson, 1981; Fennando and Andrew, 1991; Ramesh, 2001) and appeared to be a sensitive indicator of environmental stress in fishes (Li et al., 2011).

There are number of literatures available on plasma glucose level with different types of chemical toxicity in freshwater fish. For example, the existing data shows on an increase in blood glucose level under the stressful conditions of arsenic in C. catla (Kavitha et al., 2010) and C. carpio (Lavanya et al., 2011), copper in Oreochromis niloticus (Monteiro et al., 2005), crude oil in Clupea pallasi (Kennedy and Farrell, 2005), cadmium chloride in C. catla (Sobha et al., 2007), mercury chloride in C. catla (Prasath and Arivoli, 2008), nitrite in C. mrigala(Das et al., 2004), cypermethrin, copper sulfate, tannic acid and diazinon in Heteropneustes fossilis and C. carpio (Varanka et al., 2001; Banaee et al., 2008; Saha and Kaviraj, 2009) and lindane in C. carpio (Saravanan et al., 2011). Further, the pharmaceuticals drug verapamil in O. mykiss (Li et al., 2011) and CA and DCF in C. carpio (Saravanan et al., 2011b) were showed a higher level of plasma glucose content. The results of the above studies clearly envisage that the higher level of plasma glucose indicates as a secondary marker of a stress response (Toal et al., 2004).

On the other hand, a significant decrease in plasma glucose level was reported by many investigators in fish exposed to various environmental contaminants. The heavy
metals induced significant decreases in plasma glucose level in *O. mykiss* when exposed to cadmium (Chowdhury *et al.*, 2004); nickel (Pane *et al.*, 2005) and copper (Gagnon *et al.*, 2006). Similar findings were also noted in zinc exposed fish *Heteroclitus* sp (Kori-Siakpere and Ubogu, 2008), nitrite exposure in *C. catla* (Das *et al.*, 2004a) and arsenic exposed fish *C. catla* (Kavitha *et al.*, 2010). Recently, Martín-Díaz *et al.*, (2009) reported that pharmaceuticals and personal care products such as carbamazepine, caffeine, and methotrexate significantly alter the biochemical responses in the mussel *Elliptio complanata*. Further, both higher and lower level of plasma glucose was observed in various concentrations of propolis in *O. mykiss* (Talas and Gulhan, 2009).

Proteins are one of the most important and complete groups of biological materials comprising the nitrogenous constituents of the body and performing different biological functions. Interestingly, proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism. The protein is involved in major physiological events as energy precursors for fish under stress condition. Importantly, proteins exhibit functional versatility and are therefore, involved in a variety of biological roles such as transport, metabolic control, catalysis of chemicals transformation (Christian *et al.*, 1993). Protein metabolism can provide information on the general energy mobilization of an animal and show relationships with effects of contaminants in these organisms (Adams *et al.*, 1990; Simonatoo *et al.*, 2008). Catabolism of proteins makes a major contribution to the total energy production in fish (Korkmaz *et al.*, 2009). Whole body protein concentrations are influenced by a variety of environmental factors (Claybrook, 1983). Alexander and Ingram (1980) stated that the concentration of protein in the blood of fish has been used as an indicator of their general state of health. Hence, measurement of protein provides an insight on the biological mechanism of metabolism and used to understand the general state of health under stressful conditions (Keitly and Stehly, 1989; Li *et al.*, 2011; Saravanan *et al.*, 2011).

A plenty of reports are available in increased level of protein in fish exposed to various environmental contaminants. For example, Ghazaly (1992) recorded an apparent increase in plasma protein level in cadmium and mercury treated fish *Tilapia zilli*. The pesticide cypermethrin also altered the plasma protein level in an Indian major carp, *Labeo rohita* (Das and Mukherjee, 2003). Similar observation have
also been reported in *Salvelinus fontinalis* (Wood et al., 1988a); in white fish *Coregonus wartmanni* to aluminum exposure (Vuorinen et al., 1990); in *Brycon cephalus* to phenol (Hori et al., 2006) and in *O. niloticus* to copper (Abdel-Tawwab et al., 2007). Likewise, the pharmaceuticals drug propolis induced a significant increase in plasma protein in rainbow trout (*O. mykiss*) (Talas and Gulhan, 2009). Further, Li et al. (2011, 2011a) reported that the pharmaceutical verapamil and CBZ significantly influenced higher level of plasma protein content in juvenile rainbow trout (*O. mykiss*). Similar observation was also made in *C. carpio* exposed to CA and DCF (Saravanan et al., 2011b).

In contrast, Garg et al. (1989) observed a significant decline in plasma protein level during acute and sublethal manganese treatment. Similar results have been reported by many authors such as zinc and arsenic to *C. punctatus* (Hota, 1995), cadmium to *O. niloticus* (Almeida et al., 2001), chromium to *L. rohita* (Vutukuru, 2003), nitrite to *C. catla* (Das et al., 2004), copper to *O. niloticus* (Monteiro et al., 2005) and zinc to *Heterocliarias* sp (Kori-Siakpere and Ubogu, 2008). Similarly exposure of fish to various types of pesticides also induced a significant decrease in plasma protein level (Das and Mukherjee, 2003; Begum, 2005; Oruc and Usta, 2007; Sharma et al., 2009). Recently, Saravanan et al. (2011, 2011a) observed lower level of plasma protein in lindane and endosulfan exposed fish *C. carpio* and *Labeo fimbriatus* respectively. In addition to these, Kavitha et al. (2011) reported a significant decrease in plasma protein level in *Moringa oleifera* seed extract exposed fish *C. carpio* Verapamil, a pharmaceutical drug also induced a significant decrease in plasma protein level in juvenile rainbow trout (*O. mykiss*) (Li et al., 2011).

According to the previous studies, the alterations either increased or decreased level of plasma glucose and protein is depending on the type of chemical compounds, fish species, water quality and length of exposure (Vaglio and Landriscina, 1999; Monteiro et al., 2005). To our knowledge, the literature on impacts of pharmaceuticals drug on these biochemical biomarkers is scanty. Hence, the present investigation is aimed to find out the impacts of pharmaceutical drugs, CA and DCF at various concentrations on plasma glucose and protein levels in an Indian major carp, *C. mrigala* under short and long-term exposures and to use the alterations of these parameters as biomarkers against pharmaceutical drug toxicity in aquatic organisms.
MATERIALS AND METHODS

Biochemical studies

Plasma glucose and protein levels were estimated in an Indian major carp, *C. mrigala* treated with different concentrations of CA and DCF using the following methods.

Estimation of plasma glucose

Plasma glucose was estimated by *O*-Toluidine method (Cooper and Mc Danial, 1970).

Principle

Glucose reacts with *O*-Toluidine in presence of acetic acid to form a green colour derivative which is measured at 630 nm by using UV Spectrophotometer.

Reagent Utilized

- Reagent 1: *O*-Toluidine colour reagent
- Reagent 2: Glucose standard, 100 mg%

Procedure

Four test tubes were taken and marked as Blank (B), Control (C), Test (T) and Standard (S). To each test tube, 5 ml of Reagent-1 (*O*-Toluidine colour reagent) was added. Then, 0.1 ml of distilled water was added to the test tube marked B (Blank). Similarly 0.1 ml of plasma from control and CA treated fish was added to the respective tubes (Control and Test tubes). Then 0.1 ml of Reagent -2 (Glucose standard) was added to the test tube marked as S (Standard). The contents in all the tubes were mixed well and heated in boiling water for 10 minutes. Then, the test tubes were cooled under running tap water for 5 minutes and the optical density of the test samples were measured at 630 nm within 30 minutes against blank using UV Spectrophotometer. The same procedure was followed for the determination of DCF toxicity also.
Calculation

O.D. of the Test
Plasma glucose in mg/100 ml = -------------------------- x 100
O.D. of the Standard

Estimation of plasma protein

Plasma protein estimation was done according to the method of Lowry et al. (1951).

Principle

The final blue color of protein is produced by the reaction of carbamyl groups of protein molecules in the sample with alkaline copper and potassium of the reagent. This complex together with tyrosine and tryptophan of the sample is produced with phosphomolybdate of the folin phenol reagent.

Reagents

Solution A

2.00 gm of sodium carbonate was dissolved in 100.00 ml of 0.1N NaOH.

Solution B

500.00 mg of copper sulphate was dissolved in 100.00 ml of 1% sodium potassium tartarate solution.

Solution C

50.00 ml of solution A was mixed with 1 ml of solution - B.

Folin - phenol reagent

1.0 ml of folin - phenol reagent was mixed with 1.0 ml of double distilled water.

Procedure

Four test tubes were taken and marked as Blank (B), Test (T), Control (C) and Standard (S). 0.10 ml of plasma from control and CA treated fish were taken in respective tubes (Control and Test tubes). Then, 0.90 ml of distilled water was added
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to all the tubes. 1 ml of distilled water was taken in ‘Blank’ tube. They were treated with 5.0 ml of Solution-C for 10 minutes, and then 0.5 ml of Folin-Phenol reagent was added to each tube. The colour intensity (O.D) of ‘Control’ (C) and ‘Test’ (T) against ‘Blank’ (B) was read after 15 min at 720 nm by using UV Spectrophotometer.

For the preparation of ‘Standard’(S) 1.0 mg of bovine serum albumin (Sigma-Chemical company, USA) was added to 10.0 ml of IN NaOH and made up to 100.0 ml in a solution standard flask. From this, 1.0 ml of solution was taken in ‘Standard’ tube and mixed with 0.5 ml of Solution-C, kept for 10 min, and then 0.5 ml of Folin Phenol reagent was added. The optical density of the ‘Standard’ (S) was read as mentioned above. The same procedure was followed for the determination of DCF toxicity also.

Calculation

\[
\text{OD of Unknown} \div \text{OD of Known} \times \text{Concentration of Standard} = \mu\text{g of protein in ml of plasma.}
\]

3.2.2. Statistical analysis

The statistical analysis was made individually on each sample and the mean value of five individual observations was taken for each parameter. All values were expressed as means and analyzed by ANOVA, followed by a DMRT test to determine the significant differences \((P < 0.01\) and \(P < 0.05\)) among the concentrations, between the drugs, and the difference between the concentrations and drugs on each parameter. The analytical data together with Tables and Graph/Bar - diagrams are presented on appropriate places in the text for all the chapters.
RESULTS

Plasma glucose and protein levels: Short-term study

In the present study, plasma glucose was elevated at all concentrations of CA and DCF exposed fish (96 h) when compared with their respective controls (Table 10 and Fig. 13). In which, the maximum increase in plasma glucose was observed in fish exposed to 1 and 10 µg/L of CA and DCF treatments. There was a significant ($P < 0.01$) difference in plasma glucose level among the concentrations of CA and DCF, between the drugs and also between the concentrations and drugs. In contrast, plasma protein level was decreased in *C. mrigala* exposed to all concentrations of CA and DCF exposures (Table 10 and Fig. 14). In this case, a maximum decrease was noted in fish exposed to 100 µg/L of CA and DCF treatments. The concentrations of both CA and DCF, drug types and the interaction between the concentrations and drugs had a significant ($P < 0.01$) effect in plasma protein level.
Fig 13. Changes in plasma glucose level in a freshwater fish *C. mrigala* treated with nominal concentrations of CA and DCF (1, 10 and 100 μg/L; 96 h). Means in the bars followed by common letters for the drug are not significantly different ($P < 0.05$) according to DMRT.

Fig 14. Changes in plasma protein level in a freshwater fish *C. mrigala* treated with nominal concentrations of CA and DCF (1, 10 and 100 μg/L; 96 h). Means in the bars followed by common letters for the drug are not significantly different ($P < 0.05$) according to DMRT.

**Plasma glucose and protein levels: Long-term study**
During this long-term exposure, the plasma glucose level was decreased in both CA and DCF (except in 1 µg/L of CA) exposures at the end of 7th day when compared to that of their control groups (Table 11 and Fig. 15). At the end of 14th day, plasma glucose was increased in all concentrations of CA and in 100 µg/L of DCF exposed fish (Table 11 and Fig. 15). At the end of 21, 28 and 35th a significant decrease in plasma glucose was observed in 10 and 100 µg/L of CA treated fish (Table 11 and Fig. 15). However, in DCF treatment, a significant decrease in plasma glucose level was noted in 1 and 100 µg/L treated fish at the end of 21 and 28th day (Table 11 and Fig. 15). On day 35th, plasma glucose level was increased in 1 and 10 µg/L treated fish (Table 11 and Fig. 15). In these exposure days, significant ($P < 0.01$) differences were noticed in plasma glucose level among the concentrations, between the drugs and between the concentrations and drugs at the end of the study period (Table 11 and Fig. 15). However, no significant difference in plasma glucose level was observed between the drugs CA and DCF alone at the end of 21st day.

Plasma protein content of *C. mrigala* chronically exposed to different concentrations of CA and DCF were presented in Table 12 and Fig. 16. An increase in plasma protein level was noted at the end of 7th day in all concentrations of CA and 100 µg/L of DCF treated fish. After 7th day, a significant decrease in plasma protein level was observed at all concentrations of both CA and DCF treatments upto 28th day. At the end of 35th day exposure, plasma protein level was showed a mixed response with respect to the concentrations of CA and DCF. In which, 10 and 100 µg/L concentrations of CA and 1 µg/L of DCF treated fish displayed an increased level of plasma protein whereas in DCF it was decreased in 100 µg/L concentration. In this study, a significant ($P < 0.01$) variation among the concentrations, between the drugs ($P < 0.01$), and between the concentrations and drugs ($P < 0.01$) were noticed. However, no significant difference in plasma protein level was observed between the drugs CA and DCF alone at the end of 21st day (Table 12 and Fig. 16).
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Fig 15. Changes in plasma glucose level in a freshwater fish *C. mrigala* treated with nominal concentrations of CA and DCF (1, 10 and 100 μg/L; 35 days). Means in the bars followed by common letters for the drug are not significantly different (*P* < 0.05) according to DMRT.

Fig 16. Changes in plasma protein level in a freshwater fish *C. mrigala* treated with nominal concentrations of CA and DCF (1, 10 and 100 μg/L; 35 days). Means in the bars followed by common letters for the drug are not significantly different (*P* < 0.05) according to DMRT.
Environmental contaminants in aquatic media alter the physiological status of aquatic organisms which results in significant changes in biochemical parameters. The biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). The impacts of pharmaceuticals on the biochemical parameters of fish can help to understand the mechanism and mode of action of drugs (Li et al., 2011). Earlier reports on the changes in blood glucose level in response to stress are contradictory showing both a rise (Arends et al., 1999; Mzimelo et al., 2002; Das and Mukherjee, 2003; Min and Kang, 2008; Talas and Gulhan, 2009; Li et al., 2010; Li et al., 2011) and a fall (Krumschnable and Lackner, 1993; Flodmark et al., 2002; El-Sayed et al., 2007; Kavitha et al., 2010; Lavanya et al., 2011). One of the reasons behind this is that the plasma glucose is regulated by complex interactions of hormones such as glucagons and cortisol (Agrahari et al., 2007). Elevation of the blood glucose level, induced by corticoids and catecholamines, is a well-known secondary response to stress in fish (Mazeaud et al., 1977; Pickering, 1981; Vuorinen et al., 2003). Raja et al. (1992) reported that increased blood glucose may indicate disrupted carbohydrate metabolism caused by enhanced breakdown of liver glycogen, which possibly is mediated by increased adrenocorticotropic and glucagon hormones and/or decreased insulin activity. Impairment of insulin secretion may also lead to elevation of glucose and depletion of liver glycogen and an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar (Afaghi et al., 2007).

It is well known that stressful stimuli elicit rapid secretion of both glucocorticoids (Wedemeyer, 1969) and catecholamines (Nakano and Tomlinson, 1967) from the adrenal tissues of fish and both of these hormones produced hyperglycemia (Oguri and Nace, 1966; Brown, 1993). Its high concentrations in blood indicate that the fish is under stress and intensively using energy reserves, i.e, glycogen in liver and muscles (Vosyliene, 1999; Firat and Kargin, 2010). It has been reported that the increased blood glucose is usually observed in fish under undesirable conditions and it helps the animal by providing energy substrates to vital organs to cope with the increased energy demand (Velisek et al., 2006; Banaee et al., 2008; Saha and
Kaviraj, 2009; Saravanan et al., 2011). The increase of plasma glucose level indicates a stress response triggered by the stress which might be due to hypoxic condition and gluconeogenesis (Kavitha et al., 2010; 2011). However, Min and Kang (2008) suggested that increased plasma glucose levels may be a response to respiratory insufficiency due to stress.

In the present study, a significant higher level of plasma glucose in fish exposed to both CA and DCF may be due to high utilization of glucose to meet the metabolic demands caused by both drugs CA and DCF. Further, elevation of the blood glucose level may be mediated by increased adrenocorticotrophic and glucagon hormones due to drug toxicity and also as a secondary response to CA and DCF stress. The present investigation was also supported by Li et al. (2011) and Min and Kang, (2008) who noted a higher level of plasma glucose in juvenile rainbow trout *O. mykiss* exposed to CBZ and suggested that an increase in the concentration of circulating catecholamines or corticosteroids due to CBZ toxicity may results in hyperglycemic condition. On the basis of our results it is clear that pharmaceutical drugs CA and DCF acts as a stressor in fish.

In contrast, a decrease in glucose levels was noted at all concentrations of CA and DCF treated with *C. mrigala*. Vijayasree et al. (2008) reported that the drop in blood glucose in *Anabas testudineus* exposed to nitrate toxicity might be due to a high utilization of glucose for oxidation. A decreased level of plasma glucose level in Nile tilapia, *O niloticus* exposed to deltamethrin may be due to enhanced energy demand that stimulates utilization and exhaustion of glucose (El-Sayed et al., 2007). In the present study also the observed decrease in plasma glucose level during CA and DCF treatment may indicate a high utilization of glucose to cope with the enhanced energy demand cause by the drugs.

Blood protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby et al., 2006; Hadi et al., 2009). Thus, the influence of toxicants on the protein level of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy
(Hadi et al., 2009; Osman et al., 2010). Handyu and Depledge (1999) reported that changes in protein level might have arisen from protein leakage from damaged tissue, the mobilization of extracellular chelating systems, and or osmotic disturbances (e.g., dehydration). Fish under stress may also mobilize protein to meet energy requirements needed to sustain increased physiological activity (Martinez et al., 2004). Most proteins are synthesized in the liver (Burtis et al., 1996; Ziak et al., 2002) which is highly sensitive to toxicants poisoning (Prasath and Arivoli, 2008) and the higher level of plasma protein may be a result of increased production of metallothionein which is a sequestering agent (Cousins, 1982).

In the present investigation during long-term treatment of different concentrations of CA and DCF, the hepatocellular damage may be increased leading an increase in the plasma protein level and also it indicate physiological adaption to overcome stress induced by the drugs. Similar observations were also noted in blood of O. mykiss exposed to pharmaceutical drugs CBZ and verapamil (Li et al., 2011, 2011a). The increase in structural protein could be helpful to the animal to fortify its organ for developing resistance to the imposed sublethal toxic stress (Prashanth, 2007).

The liver is one of the target organs for the accumulation of xenobiotics and causes pathological alterations in fish. The significant decrease in plasma protein level in lindane and arsenic treated fish might have caused from impaired protein synthesis due to liver disorder (Kavitha et al., 2010; Saravanan et al., 2011). Jenkins et al. (2003) pointed out that a decrease in protein level may be attributed to stress-mediated mobilization of toxic compounds to fulfill an increased demand for energy by the fish. Das et al. (2004a) reported a reduction in protein level in fingerlings of C. mrigala exposed to ammonia. They further reported that the higher energy demand might have triggered an increase in protein catabolism, a process in which both blood and structural protein are converted to energy, thereby reducing protein. Further, the decrease in plasma protein may also be due to kidney disorder (albuminuria), liver cirrhosis or nephrosis or might be due to alteration in enzymatic activity involved in protein biosynthesis (Nayak et al., 2004; Palaniappan and Vijayasundaram, 2009; Lavanya et al., 2011). Furthermore, kidney damage due to toxicant stress may cause
increased renal excretion of blood protein which may contribute to the depletion of protein in the fish.

Importantly, the decline in protein content may be related to impaired food intake, the increased energy cost of homeostasis, tissue repair and the detoxification mechanism during stress (Neff, 1985; Kirby et al., 1995; Martin et al., 2010). Ghosh and Chatterjee (1989) suggested that the decrease in protein content under toxic stress may be due to: 1. Formation of lipoproteins, which are utilized for repair of damaged cell and tissue organelles 2. Direct utilisation by cells for energy requirements and 3. Increased lipolysis. In the present study the observed decline in protein level during CA and DCF treatments might have resulted from kidney disorder or liver cirrhosis due to drug toxicity. Further, the higher energy demand during drug toxicity might have triggered an increase in protein catabolism, a process in which both blood and structural protein are converted to energy, thereby reducing the protein level. The pharmaceuticals drugs propolis and verapamil induced significant alterations at lower level in plasma protein levels in *O. mykiss* (Talas and Gulhan, 2009; Li et al., 2011).

The results of the present study concluded that the different concentrations (1, 10, and 100 μg/L) of CA and DCF have a profound influence on the plasma glucose and protein levels in *C. mrigala* during short and long-term exposures. We found significant disturbances in these endpoints (significant increases and decreases of plasma glucose and protein levels). Further, the endpoints measured in the study could be used as potential biomarkers of pharmaceutical toxicity to freshwater fish in the field of environmental biomonitoring. For more detailed elucidation of the impacts of these pharmaceutical drugs CA and DCF further research is necessary along with other parameters.