4.1 Introduction

It has been recognized for many years that the haematological estimation in toxicology studies is highly significant. In the measurement of health conditions and toxicological symptoms of organisms, haematological and biochemical parameters are exercised as indicators (Thrall, 2004; Pimpao, 2007; Venkateswara Rao, 2006). While furnishing information concerning the health condition of organisms, these parameters also indicate anomalous environmental circumstances (Elahee et al., 2007). Information about the existence, status and degree of possible sickness in organisms can be promptly acquired from results of haematological and biochemical clinical assessments (Blaxhall et al., 1973). Furthermore, numerous clinical tests, such as enzyme, hormone, histopathology, microbiology etc. tests can be instrumental in estimating the intensity of hostile effects on organisms (Cazenave et al., 2005; Van der Oost et al., 2003).

In interpreting toxic effects by means of haematology, the investigator must be aware of the appropriate methods of blood collection and handling, sampling times, quality control evaluation, and test result interpretation. One should remain aware that the test substance may affect stem cell health, maturation, release, or peripheral blood cell distribution and function. Furthermore, one
must be aware of normal physiology of the animal species and its impact on haematological parameters when interpreting test results.

4.2 Selection of Parameters

Several points must be considered before selecting clinical chemistry parameters for evaluation. Some of them are sample volume requirements, information anticipated, assessment time points and sample collection method. In investigations; where clinical chemistry effects are not expected based on the known structure and/or function of the test material; a standard clinical chemistry assessment in toxicity testing is generally carried out. The parameters that would cover changes derived from all major organ systems, may be included. A conventional panel of assays includes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) for liver damage, Creatinine (CRT) to assess kidney damage. The use of more specialized tests may be justified, if the investigator suspects about the damage to a particular organ due to test material prior to onset of the experiment.

4.3 Specimen Collection and Quality

Specimen collection for clinical chemistry analysis requires the use of serum collection tubes (no anticoagulant) with or without gel for separation. Blood collected into serum separator tubes must be allowed to clot at room temperature for 30 min to minimize residual fibrin. Tubes are then centrifuged pursuant to conditions specified by the manufacturer of the serum separator
tubes. The serum is removed with a polypropylene pipette and placed in a pre-labeled screw-cap polypropylene test tube.

4.4 Haematological parameters under investigation

Blood physiology is considered as an important manifestation to the overall health status in a numerous species of fish. Haematological assessment is used as a quick investigation technique for the assessment of the health status of the fish. Thus, haematological parameters are now used when clinical diagnosis of fish physiology is pertained to determine the effects of external stressors. The application of haematological techniques in fish culture is becoming more popular and is imperative for toxicological research; environmental monitoring and fish health conditions as reported by Saliu et al., (2010). Frequently, physical and chemical variations in the surroundings are rapidly reflected as quantifiable physiological deviations in fish because of their close association with the environment. Shah et al. (2004) reported that experiments on fish blood stretches the possibility that fish blood may reveal the circumstances fish may be facing; long before there is an external symptom of diseases. Several authors have reported works on the haematological parameters of fish exposed to various toxicants (Omoregie et al., 1998; Omoregie et al., 2002; Das et al., 2003; Adeyemo, 2005; Kori-Siakpere et al., 2005; Lipika et al., 2006). Haematological indices are the important parameters for estimation of fish physiological status and they are more associated to fish existence, reproduction and growth (Moiseenko, 1998). Changes in these parameters depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases (Luskova, 1997). Deviations in
the blood parameters of fish can be used to define and endorse the
dysfunction or injuries of (organs or tissues), just like in warm-blooded
animals, in which such deviation occur because of injuries or infections of
some tissues or organs. Nevertheless, in fish, these parameters are largely
linked to the reaction of the whole organism, i.e. to the effect on fish survival,
reproduction and growth. The calculated blood indices MCV, MCH and MCHC
have a particular importance in anaemia diagnosis in most animals.

5.4.1 Haemoglobin

Haemoglobin (hb) is the oxygen-carrying pigment of the erythrocyte. It is an
oligomeric protein containing four separate globin peptide chains each of
which is non-covalently bound to a porphyrinic heme group. Each heme group
has a central iron atom that is reversibly bound with molecular oxygen. The
haemoglobin value is used to determine several of the red cell indices which
are used in exemplifying anaemia.

5.4.2 Haematocrit

The haematocrit (packed cell volume) is the reckonable quantity of
erythrocyte concentration after optimum packing of erythrocytes in a
commercially available micro-haematocrit capillary tube. The haematocrit can
also be calculated on automated haematology instruments by multiplying the
red blood cell count and the mean corpuscular volume and is expressed as a
percentage. Visual inspection of the centrifuged specimen may provide
additional information, such as confirmation of haemolysis, icterus, lipemia,
and leukocytosis.
If enhanced haematocrit is described by a haematocrit assessment that is higher than the normal value for that species and age of animal, rise in haematocrit may be attributed to either an upsurge in the circulating RBC mass or a decline in plasma volume (referring to dehydration). Decline in haematocrit is also known as erythropaenia; and might also be as a result of blood loss due to trauma, parasites, chronic disease, deficiency of iron, vitamin B-12, renal diseases, anaemia, physical agents like irradiation of marrow, an artificial heart valve, metastatic tumors, myelofibrosis or chemical agents like chemotherapy, metal toxicity, insecticides, antibiotics, tranquilizers etc.

5.4.3 Red Blood Cell Count

The red blood cell, or erythrocyte, is a non-nucleated biconcave disk shaped cells born from marrow stem cells under the influence of erythropoietin. It takes approximately five days to develop mature red blood in peripheral circulation cells from stem. Red blood cells are red only because they contain protein chemical called haemoglobin which is brilliant red in color. Haemoglobin contains the element Iron, making it an excellent vehicle for transporting oxygen and carbon dioxide. The red blood cells in body are employed to transport oxygen, carbon dioxide, and nutrients. The average life span of the red blood cell varies by species.

Although automated instruments have virtually replaced RBC counts by manual methods, the RBC count may be obtained using a haemocytometer. The RBC is usually reported in units of millions per cubic millimeter.
Anaemia, decreased RBC count, is indicated by a RBC count that is lower than the normal value for that species and age animal. Anaemia is the result of a decline in RBC mass subsequent to various mechanisms.

5.4.4 Mean Corpuscular Volume

The Mean Corpuscular Volume (MCV) estimation is the volume of the average red cell calculated from the number of red blood cells and haematocrit as described below:

\[
MCV = \frac{\text{Haematocrit (\%) \times 10}}{\text{RBC count (10^6/\mu l)}}.
\]

The presence of higher number of immature red blood cells, which are larger in size and yet not shaped biconcave; attribute to the higher MCV count generally in young animals. Red blood cells with proliferated MCV values are referred to as macrocytic. Those with declined MCV values are referred to as microcytic. The MCV is usually expressed as femto-liters (10^{-15}) or cubic micrometers.

Macrocytic anaemia may cause proliferated MVC. The macrocytic anaemia includes aplastic anaemia, haemolytic anaemia, pernicious anaemia and folic acid deficiency anaemia.

5.4.5 Mean Corpuscular Haemoglobin

The Mean Corpuscular Haemoglobin (MCH) measurement is the concentration of haemoglobin by weight in the average red blood cell (expressed in units of picograms or micro-micrograms) and is calculated as described here:
An extraordinarily high MCH blood count reading may be attributed to a few different conditions. One of the numerous conditions is known as microcytic anaemia; which occurs when the specimen has a deficiency of vitamin B-12 in the body. Another condition that may cause high MCH blood test readings is that of thyroid malfunction. Certain other vitamins and minerals may also attribute to elevated MCH blood test evaluations; if not present in the appropriate quantities.

A dwindling value of MCH indicates iron deficiency; however, an elevated value of MCH is brought about by ample haemoglobin present which may cause poor oxygen supply to the blood.

### 5.4.6 Mean Corpuscular Hemoglobin Concentration

The Mean Corpuscular Haemoglobin Concentration (MCHC) is the ratio of the haemoglobin concentration to haematocrit (expressed as a percentage) and is calculated as described below:

\[
\text{MCHC} = \frac{\text{Haemoglobin concentration (g/dl)} \times 10}{\text{RBC count (10}^6/\mu\text{l)}}
\]

This measurement is believed to be the most unchanging erythrocyte index, providing that the Hb and Hct measurements are accurate. Red blood cells with elevated MCHC values are referred to as hyperchromatic. Those with dwindled MCHC values are hypochromatic. Blood loss over time, too little iron in the body, or hypochromic anaemia may attribute to extremely low MCHC
levels. Hypochromic anaemia is another condition in which the red blood cells have a diminished amount of haemoglobin.

4.5 Biochemical parameters under investigation

The importance of appropriate clinical chemistry assessment in toxicology studies have been recognized for many years. Fish are responding to various stressors by a series of biochemical and physiological stress reactions, so called secondary stress responses comparable to those of higher vertebrates (Mazeaud et al., 1981). Sublethal effects are biochemical in origin as most toxicants put forth their effects at rudimentary level of the organism by reacting with enzymes or metabolites and other functional components of the cell. The estimation of blood chemistry parameters is a customary and imperative tool providing the vital information on the physiological status of organism (Chen et al., 2003).

For the investigator, it is mandatory to know every detail of the tests carried out, including the method and the physiological relevance of the tests, in order to decide which tests are to be carried and why. A brief discussion of biochemical parameters under investigation, Methods available for measurement and their physiological significance of each of them is presented below:

5.5.1 Alkaline Phosphatase

Alkaline Phosphatase (ALP) is composed of several isoenzymes; that are existing in almost all tissues of the body, specifically at or in the cell membranes. These enzymes have wide substrate specificity and catalyze the
hydrolysis of mono-phosphate esters. The actual natural substrates upon which they act in the body are not known. There are specific forms of ALP in liver, bone, intestine, placenta, and kidney; however, the predominant forms present in normal serum are the liver and bone forms. It is assumed that the enzyme is associated with calcification in the bone as well as lipid transport in the intestine and liver. The preferred method for analysis of serum ALP is the adenosine mono-phosphate (AMP) utilizing 4-Nitrophenyl Phosphate method that measures the 4-Nitrophenoxide ion produced by removal of the phosphate group from 4-Nitrophenyl Phosphate by ALP.

The foremost reasons of high serum alkaline phosphatase activity are stimulation of hepatic ALP (e.g. cholestasis), induction of hepatic ALP release (corticosteroids, hyperadrenocorticism), augmented osteoblastic activity in bones (hyperparathyroidism) and neoplasia (sarcoma, carcinoma).

Hepatic ALP production is provoked by enhanced intra-canalicicular (bile canaliculi) hydrostatic pressure. It is a microsomal membrane-bound enzyme that does not leak during altered hepatocellular permeability. It is the most sensitive indicator of cholestasis and will be well in advance to increases in total bilirubin. The level of the ALP elevation seen in hyperparathyroidism, bone neoplasia, rickets, or osteomalacia (from bone ALP) is not as large as that observed with cholestasis.

Exposure to numerous chemicals or drugs (e.g., acetoninophen, allopurinol, antifungal agents, halothane, clofibrate) are reported to be responsible for increases in ALP.
5.5.2 SGPT & SGOT (ALT & AST) Aminotransferases

The aminotransferases, including Alanine Amino Transferase (ALT), formerly known as Serum Glutamate Pyruvate Transaminase (SGPT), and Aspartate Amino Transferase (AST), formerly known as Serum Glutamate Oxaloacetate Transaminase (SGOT), are good indicators of damage to hepatocyte. These enzymes are present in hepatocyte cytosol and escape into the extracellular fluid, during incidents of altered plasma membrane permeability. Alanine Amino Transferase is an enzyme that catalyzes the relocation of an amino group from alanine to oxoglutarate to produce glutamate. Aspartate Amino Transferase catalyzes the transfer of an amino group from aspartate to oxoglutarate to form L-glutamate. The preferred methods for analysis of serum ALT and AST are the International Federation of Clinical Chemistry (IFCC) reference methods.

In laboratory animal species, ALT is specific for liver damage or disease, whereas AST is found in liver and muscle. As described above, when the plasma membrane permeability is altered, these enzymes leak out of the hepatocyte cytosol. The high concentration gradient between the intra and extracellular compartments is a major cause for leakage occurrence. The cause of hepatic enzyme leakage is increased plasma membrane permeability, resulting from reduced oxygen supply to the liver. Although the magnitude of the increase in ALT or AST is directly proportional to the number of hepatocytes affected, it is not related to the reversibility/irreversibility of the change. This is also true for increased AST with muscle damage or disease (e.g., myocardial infarction).
5.5.3 Creatinine

Creatinine (CRT) is derived from the non-enzymatic, spontaneous conversion of free creatinine in the muscle. Approximately 1 to 2% of muscle creatinine is converted to Creatinine daily and the amount of endogenous Creatinine produced is proportional to muscle mass. The excretion rate is also constant and equivalents production. The preferred method for analysis of serum Creatinine is the Jaffe reaction, which measures the red-orange adduct formed during the reaction between Creatinine and the picrate ion in alkaline media. There are several non Creatinine Jaffe-reacting chromogens. However, they may slightly increase the measurements.

Creatinine can deliver vital statistics about renal disease or post-renal obstruction or leakage. It is without restrictions filtered through the glomerulus; however, the renal tubules reabsorb creatinine in small amounts as well as the proximal tubules secrete it. Increased serum Creatinine levels occur when glomerular filtration is decreased. Creatinine levels are also increased by reduced renal perfusion. Creatinine clearance may also be measured and is considered to be an accurate index of glomerular filtration rate (GFR).

5.5.4 Glucose

The serum concentration of glucose is controlled by multifaceted interactions of hormones such as glucagon, insulin, cortisol, and epinephrine. The reasons of augmented serum glucose concentrations take account of hyper-adrenocorticism, moribundity, exogenous glucocorticoids and morphine.
Dwindled serum glucose concentrations can be consequent from ethanol ingestion, liver failure, and deficiency of growth hormone, glucocorticoids, and glucagon. Severe and maybe permanent central nervous system dysfunction can result from hypoglycemia. Clinical signs of hypoglycemia include confusion, lethargy, ataxia, and seizure, which may progress to loss of consciousness and death.

5.5.5 Proteins

The body contains a horde of different proteins, of which approximately three hundred can be found in the plasma alone. The majority of the plasma proteins are synthesized in the liver, with the exception of the protein hormones and immunoglobulins. They are constantly undergoing catabolism, primarily in the liver, and replacement with each plasma protein having its own specific turnover rate. The different functions of proteins are as plentiful as the proteins themselves. Different proteins serve as complement factors, coagulation factors, anions in acid-base balance, and carriers for vitamins, hormones, fats, free haemoglobin and unconjugated bilirubin. Commonly, serum total protein and albumin are the two plasma proteins that are measured. Normally, serum total protein concentration is in direct proportion to the serum albumin concentration. While hyper-albuminaemia is only seen in dehydration, hyper-gamma globulinaemia, and hyper-fibrino genaemia, hypo-albuminaemia (and hypo-proteinaemia) is common in many disease states and may result from impaired synthesis (liver disease), increased catabolism (tissue damage), reduced absorption (malnutrition), protein loss (glomerulonephritis, protein-losing enteropathy, burned skin), or altered
distribution (ascites). The preferred method for analysis of serum total protein is the biuret reaction method, which measures the amount of a colored product that is formed from the reaction of peptide bonds of proteins with copper ions in alkaline solution.

### 5.5.6 Cholesterol & Triglyceride

Cholesterol is the most important sterol occurring in animal fats. It is equally distributed between plasma and red blood cells, but in adrenal cortex, it occurs in the esterified form. The cholesterol occurs as white or faintly yellow almost odorless granules. A rise in cholesterol typically accompanies the inflammatory response and it serves to protect the nerve and brain against exposure to fat-soluble toxins and heavy metals.

A triglyceride (TG, triacylglycerol, or TAG, triacylglyceride) is an ester derived from glycerol and three fatty acids. Triglycerides are blood lipids that helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less. Triglycerides are formed by combining glycerol with three molecules of fatty acid. Triglycerides, as major components of Very Low Density Lipo-Protein (VLDL) and chylomicrons, play an important role in metabolism, as energy sources and transporters of dietary fat.

The increase in plasma triglycerides is likely due to a decrease in lipoprotein lipase activity (key enzyme in triglyceride hydrolysis). A significant increase in liver triglyceride level was also observed; this increase was attributed to increased triglyceride synthesis.
5.6 Results

Results of the chronic toxicity tests are summarised as below:

**Haematological Studies**

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>MCV (µm³)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>RBC (10⁶ mm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 Hr</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.70 ± 0.10</td>
<td>35.26 ± 1.07</td>
<td>191.19 ± 11.34</td>
<td>30.91 ± 1.63</td>
<td>16.17 ± 0.42</td>
<td>1.85 ± 0.07</td>
</tr>
<tr>
<td>0.1</td>
<td>5.33 ± 0.06</td>
<td>33.33 ± 1.03</td>
<td>201.80 ± 10.54</td>
<td>32.28 ± 1.32</td>
<td>16.01 ± 0.50</td>
<td>1.65 ± 0.05</td>
</tr>
<tr>
<td>0.2</td>
<td>5.23 ± 0.06</td>
<td>31.40 ± 1.30</td>
<td>198.32 ± 15.59</td>
<td>33.04 ± 1.85</td>
<td>16.68 ± 0.56</td>
<td>1.59 ± 0.07</td>
</tr>
<tr>
<td>0.3</td>
<td>4.90 ± 0.10</td>
<td>30.56 ± 1.32</td>
<td>204.16 ± 21.41</td>
<td>32.70 ± 2.62</td>
<td>16.04 ± 0.38</td>
<td>1.50 ± 0.09</td>
</tr>
<tr>
<td><strong>7 Days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.47 ± 0.12</td>
<td>33.50 ± 1.03</td>
<td>198.61 ± 25.16</td>
<td>32.33 ± 2.86</td>
<td>16.33 ± 0.81</td>
<td>1.70 ± 0.16</td>
</tr>
<tr>
<td>0.1</td>
<td>4.63 ± 0.15</td>
<td>30.44 ± 0.79</td>
<td>209.53 ± 6.50</td>
<td>31.93 ± 2.12</td>
<td>15.24 ± 0.87</td>
<td>1.45 ± 0.05</td>
</tr>
<tr>
<td>0.2</td>
<td>4.43 ± 0.06</td>
<td>29.02 ± 1.36</td>
<td>207.87 ± 7.65</td>
<td>31.79 ± 1.78</td>
<td>15.30 ± 0.87</td>
<td>1.40 ± 0.06</td>
</tr>
<tr>
<td>0.3</td>
<td>4.10 ± 0.10</td>
<td>26.71 ± 1.51</td>
<td>203.51 ± 17.65</td>
<td>31.29 ± 3.07</td>
<td>15.38 ± 0.83</td>
<td>1.32 ± 0.09</td>
</tr>
<tr>
<td><strong>15 Days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.10 ± 0.10</td>
<td>31.55 ± 0.63</td>
<td>188.43 ± 10.02</td>
<td>30.46 ± 1.78</td>
<td>16.17 ± 0.33</td>
<td>1.68 ± 0.07</td>
</tr>
<tr>
<td>0.1</td>
<td>4.07 ± 0.15</td>
<td>27.40 ± 1.03</td>
<td>207.72 ± 18.80</td>
<td>30.78 ± 1.81</td>
<td>14.86 ± 0.97</td>
<td>1.32 ± 0.07</td>
</tr>
<tr>
<td>0.2</td>
<td>3.60 ± 0.10</td>
<td>26.31 ± 1.10</td>
<td>223.26 ± 15.51</td>
<td>30.54 ± 1.67</td>
<td>13.69 ± 0.20</td>
<td>1.18 ± 0.04</td>
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<tr>
<td>0.3</td>
<td>3.33 ± 0.15</td>
<td>25.16 ± 0.19</td>
<td>225.59 ± 11.02</td>
<td>29.90 ± 2.22</td>
<td>13.25 ± 0.56</td>
<td>1.12 ± 0.05</td>
</tr>
</tbody>
</table>

Table 5.1: Results of Haematological studies

The present study reveals that the fish exposed to Cd showed significant reduction in RBCs, Hb and Hct. Gill et al., (1993) found a significant reduction in the RBCs, Hb and Hct in American eel (*Anguilla rostrata*) after exposure to 150µgCd/L. Karuppuasamy et al. (2005) found a significant reduction in total erythrocyte count, haemoglobin content and hematocrit value in air breathing fish, *Channa punctatus* after exposure to sublethal dose of Cd. Results of the
experiment are in good agreement with earlier works that reported a significant decrease in RBC of fresh water fish exposed to heavy metals (Vutukuru, 2005; Shalaby, 2001).

The Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) increased with increased concentrations of toxicant. Unlike the MCH and MCV value, which increased, the MCHC values demonstrated declination, with elevated concentration of cadmium.

**Bio-chemical Studies**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Concentration</th>
<th>Creatinine (mg/dl)</th>
<th>Total Protein (G/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Glucose (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hr</td>
<td>Control</td>
<td>0.14 ± 0.01</td>
<td>3.82 ± 0.44</td>
<td>404.13 ± 3.58</td>
<td>81.92 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.17 ± 0.01</td>
<td>3.78 ± 0.46</td>
<td>408.47 ± 3.00</td>
<td>86.97 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.21 ± 0.01</td>
<td>3.69 ± 0.44</td>
<td>411.80 ± 1.56</td>
<td>93.20 ± 1.73</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.23 ± 0.01</td>
<td>3.56 ± 0.48</td>
<td>413.73 ± 1.10</td>
<td>112.5 ± 2.37</td>
</tr>
<tr>
<td>7 Days</td>
<td>Control</td>
<td>0.18 ± 0.03</td>
<td>3.86 ± 0.46</td>
<td>412.00 ± 4.33</td>
<td>83.15 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.24 ± 0.01</td>
<td>3.27 ± 0.22</td>
<td>422.20 ± 0.69</td>
<td>121.00 ± 3.12</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.29 ± 0.01</td>
<td>3.11 ± 0.12</td>
<td>427.20 ± 1.91</td>
<td>134.9 ± 4.39</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.35 ± 0.01</td>
<td>2.91 ± 0.06</td>
<td>436.80 ± 1.91</td>
<td>141.6 ± 4.27</td>
</tr>
<tr>
<td>15 Days</td>
<td>Control</td>
<td>0.29 ± 0.12</td>
<td>3.90 ± 0.44</td>
<td>409.13 ± 0.75</td>
<td>82.81 ± 1.81</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.32 ± 0.01</td>
<td>3.03 ± 0.24</td>
<td>436.40 ± 2.25</td>
<td>152.2 ± 4.91</td>
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<tr>
<td></td>
<td>0.2</td>
<td>0.37 ± 0.01</td>
<td>2.88 ± 0.30</td>
<td>443.80 ± 5.37</td>
<td>162.5 ± 2.89</td>
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<td></td>
<td>0.3</td>
<td>0.44 ± 0.01</td>
<td>2.76 ± 0.30</td>
<td>447.47 ± 2.19</td>
<td>170.9 ± 2.31</td>
</tr>
</tbody>
</table>
Our study revealed that the blood serum Creatinine content increased with increasing concentration of cadmium and also with increasing time of exposure. Similar result was observed by Mona Zaki et al. (2010) in two fishes grey mullet (Mugil cephalus) and Nile Tilapia (Oreochromis niloticus).

The data of our study also demonstrated that the exposure of cadmium caused significant increase in the activities of blood serum GOT and GPT.
levels. Several researchers have already shown that these blood enzymes were highly increased in the fish treated with cadmium, zinc and copper (Benson et al., 1987; Al-Attar, A.M., 2005; Singh et al., 1990; Hilmy et al., 1987; Karan et al., 1998). De smet et al., (2000) reported that there is an upsurge in the activities of GOT and GPT in *Cyprinus carpio* exposed to cadmium. Significant rise in the concentration of AST and ALT in the liver, gill and tissues of the fish in this study is similar to that observed in Cadmium Chloride toxicity by Velmurugan et al. (2007).

Shakoori et al. (1990) reported that the upsurge in blood enzymatic activity is due to either of the reasons; namely: (i) leakage of these enzymes from hepatic cells and thus raising levels in blood, (ii) increased synthesis and (iii) induction of these enzymes. Campbell (1984) reported that these enzymes release to the blood stream when the hepatic parenchymal cells are damaged. Such variations in biochemical intensities under the effect of cadmium toxicity may result in impairment of energy requiring vital processes, and, therefore, stand as indicator about the health status of the fish population.

The present study reveals that the Serum Alkaline Phosphatase level decreases upon exposure of cadmium to *Oreochromis niloticus*, with increasing level of cadmium and duration of exposure. The decrease in activity of ALP in fish exposed to various pollutants or stressors has been reported by different researchers (Begum, 2004, Ogueji et al., 2007, Sastry et al., 1980, Goel, et al., 1982, Das et al. 2003, Rashatwar et al., 1983).
In our studies it was found that the level of triglycerides in blood serum elevated with both increase in concentration of cadmium and duration of exposure. These results are reinforced by earlier research by Al-Attar, A.M. (2005) on Nile Tilapia (*Oreochromis niloticus*). M Saeed et al. (2013) also observed similar nature of triglyceride in rainbow trout.

We found the level of cholesterol to be increasing with both increasing exposure duration as well as concentration of cadmium. Similar result has been reported by Muazzez et al., 2009, Yang et al., 2003; Sing et al.; 1990; Canli, 1995 in their experiments with different metal-exposed fishes. The results are supported by earlier research by Al-Attar, A.M. (2005) on Nile Tilapia (*Oreochromis niloticus*). M Saeed et al. (2013) also observed similar nature of triglyceride in rainbow trout.

Noteworthy lessening in protein has been reported in our experiment. Similar reduction was reported by Verma et al., 1979 in *Branchus fossils* and Vutukuru, (2005) in *Labeo rohita*.

A significant upsurge in serum glucose levels of the fish under cadmium exposure was recorded in our experiment. Also, with rising concentration of cadmium, the serum glucose level also elevated. Similar results were reported by Bedii et al. (2005), Chowdhury et al. (2004) and Almeida et al. (2001). These results are in agreement with those of Saeed (1989) and Arias (1990). Noteworthy elevation in the level of blood glucose have been reported by Benson et al. (1987), Early et al., (1990), Partap et al., (1990) and Hontela et al., (1996) in fish and rat treated with cadmium.
5.7 Discussion

The present study reveals that the fish exposed to cadmium showed significant reduction in RBCs, Hb and Hct. Although, unlike MCHC values, the Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) increased with rising concentrations of toxicant in Nile tilapia, *Oreochromis niloticus* at sublethal levels of cadmium exposure.

This may be attributed to the demolition of mature RBCs and the inhibition of erythrocyte production owing to reduction of haemesynthesis affected by pollutants, (James et al., 1999). Also, the decline in RBCs count may be attributed to haematopathology or acute haemolytic crisis that consequently results in severe anaemia in the majority of vertebrates including fish species exposed to different environmental pollutants or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in severe anemia (Wintrobe, 1978). A significant decline in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration was reported by Musa et al., (1999) in air breathing fish, *Channa punctatus* upon exposure to sublethal dose of Cd.

The perturbations in these blood indices (increase of MCV and MCH and decrease of MCHC) may be attributed to a defense against Cd toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct because of the extravagant disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to sublethal concentration of pollutants. The significant change in the MCH may be due to
the reduction in cellular blood iron, which may bring about abridged oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978). The related decline in haematological indices evidenced the toxic effect of heavy metals that affect both metabolic and haemopoietic activities.

The significant reduction in these parameters could be indication of severe anaemia caused by destruction of erythrocytes (Omoniyi et al, 2002 and Kori-Siakpere et al., 2009); Haemodilution resulting from impaired osmoregulation across the gill epithelium (Adeyemo, 2005 and Ayuba 2008) and it could be as a result of the destruction of intestinal cells. Gaafar et al., (2010) reported that prolonged reduction in haemoglobin content is detrimental to oxygen transport and disintegration of the erythrocytes; may be due to hostile pathological condition in fish exposed to toxicants.

Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as a good indicator of anaemia (Blaxhall et al., 1973). The declined haemoglobin in the experimental fish exposed to cadmium might thus be a suggestion that anaemic condition become apparent in the fish during exposure. Declined haemoglobin subsequent to metal exposure usually results in haemodilution. This haemodilution has been looked upon as a mechanism that decreases the concentration of the toxicant pollutant in the circulatory system (Smit et al., 1979). The observed reduction in the haemoglobin and haematocrit values in the fish may be caused by the lysing of erythrocytes. Similar observations have been reported by Samprath et al. (1993) and Musa et al., (1999) when they exposed fish
to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters is an indication of severe anaemia.

Haematocrit is used to conclude the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood (Larsson et al., 1985). The cause for the decline of circulating erythrocytes of stressed fish has been attributed to accumulation of red blood cells in damaged gills (Singh et al., 1982). Erythropaenia might also be as a result of damaged gills and impaired osmoregulation during sublethal cadmium exposure, which causes haemodilution leading to a decrease in the number of red blood cells through haemolysis (Wedemeyer et al., 1981). The noteworthy reduction in the haematocrit in this study might be due to gill damage and impaired osmoregulation causing anaemia and haemodilution.

The red blood cells have the important function of haemoglobin transport which carries oxygen to all tissues in the body. The dwindled red blood cell number subsequent to exposure to cadmium may possibly be as a consequence of haemolysis or destruction of the red blood cells. Reductions in the red blood cells can also be due to internal bleeding caused by damaged kidney.

The present investigation verified that the *Oreochromis niloticus* exposed to sublethal concentrations of cadmium demonstrated a significant rise in the amount of blood glucose after the exposure periods. Elevated levels of blood glucose are triggered by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue resulting in increased level of circulating
glucocorticoids and catecholamines. Both of these produce hyperglycaemic condition. Hyperglycaemic response in this study is an indication of a disrupted carbohydrate metabolism possibly due to enhanced breakdown of liver glycogen. Wedemeyer et al. (1981) reported that the elevation in the blood glucose level is reaction to the increased rate of glycogenolysis or gluconeogenesis (Sastry et al. 1985).

Such increase of glucose might be attributed to a number of reasons and one of them is the decline in the specific activity of some enzymes like phosphofructo-kinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (Almeida et al., 2001). The same author also found that heavy metals escalate the glucose content in blood, as a result of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids. It has been already recognized that, cadmium modulate the metabolism of carbohydrates, bringing about hyperglycemia by stimulating the glycogenolysis in some marine and fresh water fish species (Zikic et al., 1997; Levesque et al., 2002).

The experimental decline of plasma protein might also result from the breakdown of protein into amino acids and perhaps into nitrogen and other elementary molecules. The gills become “leaky” to water and ions, often causing osmoregulatory imbalances, when exposed to stressors. Therefore, the decline in serum total protein might have been attributed to several pathological processes including plasma dissolution, renal damage, protein elimination in the urine, a decrease in liver protein synthesis, alteration in hepatic blood flow and/ or hemorrhage into the peritoneal cavity and intestine.
Decline in serum protein amount could be endorsed to renal excretion or compromised protein synthesis or due to liver disorder (Kori-Siakpere, 1995). The decline in plasma and tissue protein may take place due to the upsurge of protein breakdown as a result of stimulated corticosteroid hormones, which enhance the breakdown of proteins to provide amino acids and gluconeogenesis to provide glucose to compensate for increase in energy demands under stressful condition. The running down of total protein content may be owing to breakdown of protein into free amino acid under the effect of stressor at the lower exposure period (Shakoori et al., 1994). Decline in protein level may be due to inhabitation or stimulation of metabolizing enzymes by administration of toxicants. The quantity of protein is dependent on the rate of protein synthesis as well as on the rate of its degradation. The quantity of protein may also be affected by impaired incorporation of amino acids into polypeptide chains. The reduction in protein intensities may be due to their degradation and also to their possible utilization for metabolic purpose. Bradbury et al., (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The observed hyperproteinaemia may be attributed to either of the following reasons: (i) water loss in the serum, (ii) relative change in mobilization of protein, (iii) elevated de novo synthesis (Hilmy et al., 1986; Gopal et al., 1997 and Ruparelia et al., 1989).

Cholesterol concentrations in the serum of metal-exposed fish generally increased, as compared to the control value (Muazzez et al., 2009). The
Concentrations of cholesterol is an essential structural components of membranes and the precursor of all steroid hormones, may increase due to the liver failure; causing the release of cholesterol into the blood. Hypercholesterolemia observed may be due to impairment of liver and inhibition of enzymes, which converts cholesterol into bile acid (Murray et al., 1990). Reduced lipoprotein lipase activity plays a role in the increment of plasma lipid (Asha Agrawal et al. 1999). The increased levels of cholesterol develop weakness in the body and swimming ability of the fish was observed to decrease in our study.

The elevation in triglyceride level in blood serum has already been reported by number of researchers. The same has been observed in different animals with exposure to different metal pollutants. Such elevation may be by reason of the influence of the pollutants on thyroid function (Kawada et al. 1980; Kirubagaran et al., 1994). Hyperthyroidism induced by cadmium can lead to dysfunction of liver and in turn can affect triglyceride metabolism.

Several reporters showed that blood enzymes such as SGPT (Serum Glutamate Pyruvate Transaminase) and AST (Aspartate amino Transferase) were highly increased in the fish treated with cadmium, zinc and copper (Benson et al. 1987; Al-Attar, A.M. 2005; Singh et al. 1990; Hilmy et al. 1987a; Karan et al., 1998). As mentioned earlier in this chapter the increase in blood enzymatic activity is either due to leakage of enzymes from hepatic cells, increased synthesis or enzyme induction of these enzymes. The increased serum transaminase (AST) may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation (Daabees et al.,
1992). The tissue damaged by toxicants exhibit a sharp rise in activity of mitochondrial enzymes Aspartate amino transferase (Abdel-salam et.al. 1982, Mikhail et.al. 1979). Aspartate amino transferase has been strongly implicated in the production of energy in tissues (Srivastava et.al. 1999) and is considered as a stress indicator (Gould et.al. 1976). Aspartate amino transferase is the main transaminase that interferes with TCA cycle in a major way (Lowenstein 1967, Rao et al. 1984, Al-Attar 2010). A rise in its activity indicates the occurrence of greater energy demand which is normally associates with synthetic activities of the cell (Meister 1955). The intoxication of cadmium combine with an enzyme to form an enzyme inhibition complex, which react with various functional groups of the enzymes inhibit the enzyme activity of major metabolic site (Joseph et al. 2011). Serum level of Aspartate amino transferase become elevated may be due to hepatotoxicity of liver cells. These enzymes are biomarkers to know the intensity of liver damage.

The significant changes in activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James et al., 1991 and Svoboda 2001). The increase in concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs (namely liver). In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation by these heavy metals, perhaps in liver, heart or muscle.

Creatinine level is indicator of kidney function. In the present study, Creatinine showed a significant increase in fish exposed to Cadmium. These may be due to the action of heavy metal on glomeruli filtration rate (Abbass et al. 2002)
and cadmium may cause pathological changes to the kidney resulting in dysfunction. Thus elevated Creatinine level may be indicative of renal insufficiency.

Exposure of cadmium induced a significant rise in blood triglyceride level in the fish. This may be due to decreased thyroid secretion (hypothyroidism), nephrosis, metabolic disorders associated with endocrine disorders and liver dysfunction as the liver is the principal center of lipid metabolism.

The decreased activity of alkaline phosphatase indicates disturbances in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Depletion of enzymes in the fishes exposed to cadmium observed in the present study can be attributed to increased cadmium levels in the tissues. Furthermore accumulation of cadmium in liver and muscles could be the possible reason for variation of enzyme activities. The inhibition in protein level may also be due to the decrease in alkaline phosphatase activity as it plays an important role in protein synthesis (Pilo et. al., 1972).

The present study showed that cadmium altered the entire biochemical metabolism in Oreochromis niloticus by changing the levels of Total glucose, total protein and total cholesterol, AST, Alkaline Phosphatase, Triglyceride, Creatinine and GPT in serum. Such changes in biochemical levels under the effect of cadmium toxicity might results in impairment of energy requiring vital processes, and hence give an idea about the health status of the fish population.
5.8 References


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Ch.5 Chronic Toxicity Studies


