CHAPTER – III

Immuno-Haematology
HAEMATOLOGY

INTRODUCTION

Aquaculture is one of the fastest growing aspects of agriculture industry worldwide to meet the food demand (FAO, 2001). Among biological changes, haematological parameters are considered potential biomarkers of exposure to chemical agents, since the latter can induce an increase or decrease in the various haematological components (Heath, 1995; Van der Oost et al., 2003). The effects of pesticides have been observed in blood parameters of fishes.

Insecticides are one such category of organic pollutants which play an important role in controlling different types of insects/pests that cause damage to crop plants. Unfortunately, most of the insecticides are not biodegradable and tend to persist for years together in soil and water (Gaafar et al., 2010).

Lihocin (an organochlorine insecticide) is a broad spectrum insecticide used to control insect's pests of rice, cotton, soyabean etc. These insecticides by their easy entry into the aquatic ecosystem (as runoff) may also result in damage of non target organisms particularly fishes. Determination of extent of damage to different body system viz., respiration, feeding, osmoregulation and reproduction including blood (Du Preez and Van Vuren, 1992) exposed to different xenobiotics therefore become very important. Among different systems haematology acts as an essential index of the general health status of the fish (Larsson et al., 1985).

Lihocin is commonly used insecticide in crop field and its bioaccumulation is known to cause impairment in various physiological processes under the conditions of long term exposure.

Fish haematology is an effective aid in the diagnosis of fish stress and disease. In addition, blood is the most accessible part of the teleostean body fluid system (Culloty et al., 2002). As a result, blood variables are commonly used as direct or inferential indicators of functional state. Blaxhall and Daisley, (1973) used measurement of haematological status to evaluate dietary adequacy. Common haematological parameters used are haemoglobin and haematocrit levels because they are simple to measure and are also sensitive to stress (Mathiessen, 1981; Carusco
et al., 1991). In 1981, Mathiessen reported on the haematological changes in Tilapia spp, and Clarias spp.

The use of synthetic pesticides has increased with the growing awareness about their utility in agriculture production, animal husbandry and postharvest technology and in the public health and welfare of mankind. The intensive use of synthetic pesticides in agricultural fields and public health operation systems has resulted in serious environmental hazards (Singh et al., 2004; 2006). Due to their long term persistence, slow degradability in the water, toxicity to other organisms (Arasta et al., 1996) and accumulation inside the fish body, synthetic pesticide adversely affect the aquatic environment (Cullen and Conneil, 1992; Waliszewski et al., 1999).

To minimize environmental pollution by pesticides, efforts are being made to find pesticides from plant origin because plants are virtually inexhaustible source of structurally diverse and biologically active substances (Mian and Mulla, 1992; Istvan, 2000).

A large number of biologically active compounds of various classes have been tested for insecticidal, piscicidal and molluscicidal activities (Singh et al., 2004; Selvarani and Rajamanickam, 2003; Srivastava et al., 2003; Singh et al., 2010; Barky, 2009). In fishes, a change of the blood cell distribution is also correlated with the changes in environmental conditions (De Wilde and Houston, 1967; Adeyemo, 2005; Omoniyi et al., 2002). The need to produce food in large quantities to cater for the ever increasing human population in the developing parts of the world has led to increase in the use of agrochemicals (fertilizer and pesticides). Pesticides are used to control pests of food crops, livestock and human health.

Due to their injudicious and indiscriminate usage, water bodies like ponds, lakes and low lying water filled areas are continuously polluted (Kumar and Saradhamani, 2004). Exposure to low level of pesticides may have profound effects on non-target organisms. Most pesticides enter into the food chain and cause physiological damage (Abdul Naveed, 2003; Waliszewski et al., 2003) and may interfere with the endocrine system (Kira, 2000; Min, 2000). Silva and Gammon (2009) also reported that disruption of the endocrine system by endosulfan occurs only at doses that cause neurotoxicity.
Injudicious and indiscriminate use of agrochemicals have caused great among health and environmental scientists because records of field application of pesticides even in developed countries revealed that less than 0.1% of pesticides applied to crops reach target pest, thus over 99% moves into ecosystem to contaminate the land, water and air (Pimentel and Levitan, 1986).

One of the ways to detect changes in the physiology of organisms is through analysis of the blood samples. Haematological profile of an animal gives a clear insight into the effect of environmental stressors and blood being a medium of intercellular and intracellular transport which comes in direct contact with various organs and tissues of the body. Alteration in erythrocyte indices had been reported in fishes subjected to environmental stressors like pesticides and heavy metals (Gill and Pant, 1987; Bhatia et al., 2004; Johal and Grewal, 2004).

Blood functions as a transport and distribution system for the body, delivering essential nutrients to tissue and at the same time removing waste products. It is composed of an aqueous solution containing molecules of varying sizes and a number of cellular elements. Some of the components of blood perform important roles in the body’s defense against external insult and in the repair of damaged tissues. (Baynes and Dommiczak, 2005). Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organ (Bayness and Dommiczak, 2005).

The cells of the tissue of the body are in contact with body fluids which in turn are in equilibrium with the fluid portion of the blood. Hence haematological studies are important diagnostic tools in medicine. However recent speculations have proved that may be used as valuable indicators of disease or stress in animals (Calabrese et al., 1975). Haematological values are widely used to determine systematic relationship and physiological adaptations including the assessment of general health conditions (Alkinson and Judd, 1978).

Haematological indices vary from animal to animal and in some animals at different stages of life. At birth the haemoglobin content is higher during any other stage of life. The erythrocyte count and hemoglobin concentration gradually rise in the adult levels by the time of puberty with a characteristic low level in females than in males (Hawkins et al., 1954; Vanlquist, 1950).
The importance of the haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). Blood parameters are probably the more rapid and detachable variations under stress and are full in assessing the health condition (Hymavathi and Rao, 2000). In human medicine, investigation of haematological parameters is necessary for clinical diagnosis of a disease and pathological condition (Hardiker and Gokhale, 2000). Blood is the only variable tissue in occupationally exposed workers and it is a pathophysiological reflector of the whole body, hence blood parameters are routinely used for diagnosing and monitoring the disease conditions in humans (Rahman and Sidiquie, 2006).

Blood is the most important body fluid that governs vital functions of the body. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals (Calabrese et al., 1975). For many years the study of haematological parameters has been used as diagnostic tool to investigate disease and physiological and metabolic alterations (Bansal et al., 1979). Haematological values are widely used to determine systemic relationship and physiological adaptations including the assessment of general health conditions (Alkinson and Judd, 1978).

Age related hematological changes in cotton rats (Sigmodon hispidus) were reported by Maity and Guru (1998). RBC, Hb and Haematocrit increased gradually in both sexes up to the age of 120 days and then decreased, where as the values of WBC increased gradually up to age of 60 days.

In mammals and other chordate taxa the haematological parameters are reported to vary with sex (Sealander, 1964; Srihari and Shakuntala Sridhara, 1986; Tos - Luty et al., 2001; Haratym-Maj, 2002), age (Maity and Guru, 1998), body weight (Pandey, 1977), size Pradhan, 1961), nutritional status (Smith, 1968) season (Natvig et al.,1963; Sealander, 1964), habitat (Haws and Good Night, 1962) and altitude (Murray et al., 2007).

Apart from these factors, pesticides, drugs metals (Jadwiga Chmielnicka et al., 1994; Hymavathi and Rao, 2000; Suricuchi et al., 2000; Vasantha Sena, 2002; Mughani et al, 2003 Othman 2004; Sharma et al.,2006), dyes (Mathur et al.,2003; Ramulu et al., 2006), industrial effluents (Meenakala, 1978), chemical-dimethyl
formanide (Lynch et al., 2003) and parasites infection (Arti Saxena and Shakuntala Shukla, 2006; Krishnan, 2006) also cause alternation in the haemotological parameters.

Fish is very important to humans because it contains protein of very high quality and also has sufficient amounts of all the essential amino acids required by the body for growth and maintenance of lean muscle tissue. The protein in fish as well as similar foods of animal origin makes up complete protein sources in many people's diet around the world. High quality proteins, such as protein in most fresh fish, can be used to maintain an active metabolism. Low quality protein does not the protein must either amino acids required for the use in protein synthesis and means the protein must either be used for energy or converted to fat.

Fish is one of our most valuable sources of protein; about 25% of animal protein is obtained from fish and shell fish. About 35% of all fish is eaten fish, chilled and frozen. It is also cured or canned (16%) or made in to oil and fish meal (32%). Fishes are used as medicine ground into vitamins or processed in to cosmetics and perfumes, lubricants above uses scientists often use fish, especially gold fish, for experiments and medical research. Fish are rich in omega - 3 fatty acids which plays very important role for normal growth particularly for the blood vessels and the nerves as well as keeping our skin and other tissues youthful. Research studies have revealed that in populations that consume large quantities of fish, with a high utilization of Omega 3s, there is a reduced risk of heart disease. Fish is important in diets and livelihoods of many poor people suffering from vitamin C and mineral deficiencies (Toft, 2001).

The national fish demand is about 1.85 million tons while the local production is only 0.51 million tones, based on population figure of 140 million people. India currently imports 0.7 million tons of frozen fish annually making it the highest importer of frozen fish in the world with annual foreign drain of 35.0 billion $ the challenge therefore is to bridge the wide gap between fish demand and supply.

In order to meet the growing demand of fish in aquaculture industry is growing carp fish are the primary species produced at domestic fish farms, but it will be quite some time before production can match consumer demand. Aquaculture the act of fish culture has existing for a long and for over two decades, domestic supply of fish has been in adequate. Aquaculture is the least exploited, the only option left is
to practice aquaculture farming. But one of the major problems faced by the aquaculturist today is the provision of nutritive and cheap feed to reduce cost of production.

The reduction of toxic elements like OC pesticides in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation. Chemical can effectively remove certain toxic elements from industrial wastes or polluted media, but is usually costly. However, there are some the remobilization of pesticides by synthetic anthropogenic chelating agents has received.

In a healthy state, fish defend against potential invaders with a complex system of innate and adaptive immune mechanisms. Inspite of limited pathogen recognition machinery, the strength on innate defence mechanisms against biotic and abiotic stressors is impressive. Stressors may directly kill the fish or indirectly exacerbate diseased state by lowering the resistance and allowing the invasion of environmental pathogens. Exposure to individual stressors may affect the immune system in a variety of ways - altering macrophage function (Beaman et al., 1999; Duffy et al., 2003) and circulating levels of immune cells.

The simplicity of determining certain blood parameters is probably responsible for the rise in the use of haematology as a tool for testing of health problems in fish, even those not usually directly associated with the blood. In medicine, haematological techniques are used for the following three objectives:

1) Differentiating normal from abnormal blood,
2) Diagnosis of disease or abnormality,
3) Detailed studies in haematology

Pathological conditions in fish are manifested in the form of changes in the behavior (symptoms) and/or in the integrity of the tissues (lesions), leading to a decrease in weight gain and often, death.

The study of the physiological and haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly in regard to the use in detection of healthy from diseased or stressed animal. The count of red blood cells is quite a stable index and the fish body tries to
maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters. Blood cell responses are important indicators of changes in the internal and/or external environment of animals.

In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases. Like in warm-blooded animals, changes in the blood parameters of fish, which occur because of injuries of the latter organs or tissues, can be used to determine and confirm the dysfunction or injuries of the organs. However in the fish, these parameters are more related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth. It should be noted that although the mechanisms of fish physiology and biochemical reaction to pesticides has not been investigated enough, it is obvious that species differences of these mechanisms exist.

Despite the tremendous progress of research work on pesticide toxicity in fish the work on haematological changes under pesticidal impact appear to have been marginally dealt with. Therefore the present investigation is undertaken to elucidate the effect of Lihocin, A. veronii and treated with silver nanoparticles on certain haematological parameters in fresh water fish Catla catla.
RESULTS

The present study has indicated that the fish *Catla catla* exposed to 1/5th sublethal concentration of Lihocin showed a significant and gradual reduction in TEC, Hb and PCV than those of control animals for 3, 7, 15, 30 and 45 days (Table - 3.1; Figures: 3.1 - 3.4). It is obvious that the haematological parameters can serve as an index to evaluate the level, rate and impact of pesticides in fishes.

TOTAL ERYTHROCYTE COUNT

It is obvious from the results that total TEC count was found significantly decreased in Lihocin treated groups from day 3 to day 45. Compared to control (Table -3.1; Fig- 3.1). The calculated percent changes decreased are 14.84, 20.00, 33.19 and 44.74, after 3, 7, 15, 30 and 45 days respectively (Table- 3.1).

![RBC](image)

**FIGURE-3.1:** Variations in Total Erythrocyte Count (TEC) of fish, *Catla catla* treated with sub-lethal Concentration of Lihocin, *Aeromonas veronii* and AgNPs.

The TEC count was more significant decrease in *Catla catla* treated with Lihocin and *Aeromonas veronii* for over 45 days and the Percent decreases are 44.37, 48.65 and 52.98 for 15, 30 and 45 days respectively over the control.
It is also evident from the results that the percent decrease of TEC count was gradually recovered when the fish also treated with Lihocin, *A. veronii* and silver nanoparticles (AgNPs). The percent decreases are, 51.75, 47.00 and 40.61 for 15, 30 and 45 days respectively (Table - 3.1; Fig- 3.1). ANOVA has indicated that the percent variations are gradually recovered when fish treated with AgNP.

**TOTAL HAEMOGLOBIN CONTENT**

Figure- 3.2 indicates that the Lihocin treatment caused a significant decrease in Hb contest for over 45 days. The percent decreases in Lihocin treated groups for 3, 7, 15, 30 and 45 days are 10.49, 12.46, 14.91, 24.18 and 36.92 respectively, when compared to the control group (Table- 3.1; Fig -3.2).

![FIGURE-3.2: Variations in Total Haemoglobin Content (Hb) of fish, *Cuta catla* treated with sub-lethal Concentration of *Lihocin, A. veronii* and AgNPs](image)

It is also evident from the results that the maximum percent decrease was recorded on day 45 treated with Lihocin.

ANOVA has also indicated that the significant variations among mean values at different significant levels. Table - 3.1 also indicates a significant decrease in Hb content treated with Lihocin and *Aeromonas veronii*. The percent changes are 38.15, 41.62 and 42.37 for 15, 30 and 45 days respectively.
Figure - 3.2 also indicated that the percent decrease was recovered when the fish were treated with Li+AV+AgNPs for over 45 days period. The recovery percentages are 37.67, 33.31 and 29.42 were 15, 30 and 45 days respectively.

**HAEMATOCRIT (Hct) (OR) PACKED CELL VOLUME (PCV)**

Haematocrit is also followed the similar trend to TEC and Hb significantly. Haematocrit is also gradually and significantly decreased with increase in exposure period of 1/5th sub lethal concentration of Lihocin (Table - 3.1; Fig - 3.3).

The percent changes in haematocrit are for 3, 7, 15, 30 and 45 days of 1.43, 15.23, 16.88 18.78 and 30.09 respectively.

The percent decrease was more pronounced with treatment of (Li+Av) Lihocin and *Aeromonas veronii* and the percent decreases are 36.50, 41.75 and 49.09 for 15, 30 and 45 days respectively. The maximum percent decrease was observed on day 45.

It is also evident from the results that the percent decrease was gradual decrease, when the fish treated with silver nanoparticles (C+ Li+ AV+ AgNp). The percent variations are 38.58, 28.66 and 23.55 for 15, 30, 45 days respectively.

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\text{FIGURE-3.3: Variations in Total Haematocrit (Hct) of fish, *Catla catla* treated with sub-lethal Concentration of Lihocin, *A.veronii* and AgNPs}
\]
ANOVA has been indicating that the variations are significant among mean values at 0.05 ($P < 0.05$).

**TOAL I. EUCOCYTE COUNT (TLC) (or) WHITE BLOOD CELI.I. COUNT (WBC)**

It is evident from the result that the total leucocyte count of *Catla catla* showed a gradual increase with increase of Lihocin treatment for the period of 45 days (Table-3.1; Fig -3.4). The percent increase in total leucocyte count is $5.54$, $7.84$, $12.96$, $15.08$ and $33.38$ for $3$, $7$, $15$, $30$ and $45$ days respectively.

ANOVA has been indicating that the variations of the significant among mean values at different significant levels. It is also obvious the percent increase in TLC was more pronounced with the treatment of *Aeromonas veronii* (C+Li+AV).

![WBC](attachment:image)

**FIGURE-3.4:** Variations in Total Leucocyte Count (TLC) of fish, *Catla catla* treated with sub-lethal Concentration of Lihocin, *A.veronii* and AgNPs

The percent increase was gradually decreased with an increase in the exposure periods under the treatment of silver nanoparticles (C+ Li+ AV+ AgNPs) for over 45 days period (Table -3.1; Fig -3.4). The percent decrease in TLC is $80.77$, $50.96$ and $30.51$ for $15$, $30$, and $45$ days respectively.
It is predicted that with increase in the Lihocin treatment exposure periods the fish undergo oxidative stress resulting the production of more numbers of leucocytes indicating the increase of immunity to sustain the animal in the toxic environment.

Table-3.1: Variations in Total Erythrocyte Count (TEC), Total Haemoglobin content, Packed cell volume and Total Leucocyte count of fish, *Catla catla* treated sub-lethal concentration of Lihocin, *A. veronii* and AgNps.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C+Li</th>
<th>C+ Li + AV</th>
<th>C + Li + AV + AgNp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Control</td>
<td>3d</td>
<td>7d</td>
</tr>
<tr>
<td>TEC (10^6 x mm^-3)</td>
<td>4.85 ± 0.14</td>
<td>6.15 ± 0.12</td>
<td>3.08 ± 0.15</td>
</tr>
<tr>
<td>% change</td>
<td>——</td>
<td>(-14.84)</td>
<td>(-10.00)</td>
</tr>
<tr>
<td>Hb (gdl^-1)</td>
<td>14.68 ± 0.17</td>
<td>13.14 ± 0.35</td>
<td>12.85 ± 0.22</td>
</tr>
<tr>
<td>% change</td>
<td>——</td>
<td>(-18.49)</td>
<td>(-12.46)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>32.16 ± 1.32</td>
<td>27.84 ± 1.18</td>
<td>27.23 ± 1.15</td>
</tr>
<tr>
<td>% change</td>
<td>——</td>
<td>(-15.23)</td>
<td>(-16.00)</td>
</tr>
<tr>
<td>TLC (10^6 x mm^-3)</td>
<td>18.49 ± 4.32</td>
<td>149.17 ± 5.49</td>
<td>149.36 ± 2.82</td>
</tr>
<tr>
<td>% change</td>
<td>——</td>
<td>(-5.54)</td>
<td>(-7.84)</td>
</tr>
</tbody>
</table>

- Values are mean ± SD of 6 individual observations.
- All values are significant at P< 0.05 by ANOVA.
DISCUSSION

Pesticides are known to alter the blood parameters of fishes. A significant decrease in RBC, Hb content and Haematocrit has been observed earlier, in fishes exposed to different pesticides (Palanisamy et al., 2011; Park et al., 2004). The findings of the present investigation also revealed a similar decrease intend in all the parameters such as RBC, Hb and haematocrit suggesting the organochlorine pesticides induce changes which give evidence for decrease haematopoeisis, followed by anemia induction in test fishes (Seth and Saxena, 2003).

The decreased erythrocyte content and haemoglobin content observed in the present study may be due to the disruptive action on the erythropoietin tissue which in turn affected the cell viability.

In the present study it is observed that there is an increase in WBC count which can be correlated with an increase in antibody production, which helps in survival and recovery of fishes exposed to the toxicant (Ramesh and Sarvanan, 2008). A significant increase in WBC count in the present study indicate a hypersensitivity of leucocytes to Lihocin and these changes may be due to immunological reactions to produce antibodies to cope with stress induced by Lihocin.

In fishes a change of blood cell distribution is also correlated with the changes in environmental conditions (De wilde and Houston, 1967; Gardner and Yevich, 1969). The exposure of Catla catla to sub lethal concentration of Lihocin caused a significant decrease in Erythrocyte count, Haemoglobin and Hematocrit of fish. The decrease in haemoglobin concentration is similar to those reported in C. glareipinus exposed to cassava effluents and tobacco. (Nicotiana tabaccum Linn) leaf extracts (Adeyemo, 2005; Omoniyi et al., 2002). This pattern of response may be attributed to haemolysis which results in haemodilution, the means of diluting the haemoconcentration of the extracts, thus reducing the effect of the toxicants (pollutants) in its system.

This effect on fresh water fish Catla catla might have been achieved through failure or separation of normal mechanism promoting erythropoeisis and/or deficiency of some factors required the maturation of the red cell. The causes of leucopenia observed in the present study are supposed to be according to the
degeneration, depletion and destruction of the blood forming materials by this compound. The observed depiction in the haemoglobin and haematocrit values of fish could also be attributed to the lysing of erythrocytes (Ovi and Oghoghene, 2008). Thus the significant reduction in these parameters is an indication of severe anemia.

The white blood cells in fish respond to various stressors including infections and chemical irritants. Thus increasing or decreasing numbers of white blood cells are a normal reaction on the exposure of toxicants (Kori - Siakpere, 2006). In the present investigation the increase in WBC (leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of toxicant (Gabriel et al., 2009).

Changes in total erythrocyte count of control and fishes treated with Lihocin are depicted in Table-3.1. Comparison of data of control with that of the treated groups very clearly indicated that there is a mark decline TEC of the fishes in all the tested groups. One way ANOVA results reveal that the changes in TLC were highly significant (P < 0.01) at all intervals of Lihocin exposure in all the treated groups. Similar decline has also earlier been reported in the fishes by Das and Mukherji (2000a); Verma (2007); Raina (2012) following exposure to different xenobiotics.

Decline in TEC present author also feels seemingly appears to be due to combined effect of haemolysis of RBC’s and malfunctioning of haemopoietic organs. Decline in TEC also appears to the outcome of:

(1) An increase in the rate of erythrocyte destruction due to their lysis.
(2) Reduce surface area of RBC due to their abnormal shapes.

The microscopic examination of smear preparations of blood of *Catla catla* indicates the distorted shape of erythrocytes (Fig- 3.1), which present author feels, may cause an imbalance in the respiratory physiology of the fish by reducing the surface area of haemoglobin and its access to oxygen. It can, therefore, be very safely inferred that Lihocin has induced conspicuous alterations (both qualitatively and quantitatively) in TEC of *Catla catla*.

Similar to TEC, Hb and Hct also exhibited similar decline. In the present study the author proposed that the decline in Hb Seemingly appear to cause rapid oxidation of haemoglobin to methaemoglobin and (or) release of oxygen free radical.
Free radical of oxygen by causing haemolysis may lead to reduced oxygen carrying capacity of blood. Prolonged reduction in Hb content may be deleterious to the oxygen transport.

The findings of the present investigation also reveal a similar decreasing trend in all the parameters such as RBC, Hb content, and haematocrit suggesting that the organo pesticides also induce changes which give evidence for decrease haematopoiesis followed by anemia induction content observed in this study may be due to the disruptive action on the erythropoietic tissue, which in turn affected the cell viability. The increase in WBC count can be correlated with an increase in antibody production, which helps in survival and recovery of the fishes exposed to the toxicant (Seth and Saxena, 2003).

A significant increase in WBC count in the present study indicate a hypersensitivity of leucocytes to monocrotophos and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by Monocrotophos (Ramesh and Saravanan, 2008).

The alterations of the haematological parameters would be used as an important tool for the assessment of pathological conditions of animals. The changes in the haematological Parameters of fish are a helpful biomarker for evaluating their health status (Rehulka et al., 2004).

The Lihocin induced reduction in the blood parameters recorded in the present study. This may be due to haemolysis and/or on haemorrhage caused actions of pollutants to fish (Simonato et al., 2008).

In the present study the haematological parameters are negatively corrected with the Lihocin concentration and the experimental length. The results agreement with (Jenkins et al., 2003) stated that the reduction in the haematological parameters after sub lethal Lihocin exposure is the result of the inhibition of erythropoietin, chemosynthesis, osmoregulatory dysfunction or an increase the rate of erythrolysis. Although the level of hemoglobin reduced in *Catla catla* exposed to Lihocin, the erythropoietin could be associated with a possible haemolysis (Drastichova et al., 2004). Banik et al. (1996) reported that *Anabos testdineus* exposed to 0.00125% of endosulfan are unable to absorb iron efficiently from the intensive leading to decrease
in the haemoglobin formation and then anemia. On the other hand the reduction in erythrocyte numbers could be related to possible oxidative damage to haemoglobin caused by the lipid peroxidation observed in fish exposed to sub lethal concentration of endosulfan (Jenkins et al., 2003).

A reduction in haematological values indicated anemia in the pesticide exposed fish may be due to erythropoietin, haemosynthesis and or osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs (Ogundiran et al., 2009).

In the present study decrease in RBC count during the chronic treatment might be resulted from severe anemic state or haemolysing power of toxicant Lihocin particularly on the red cell membrane. Pesticides were found mainly in the erythrocytes (particularly in the erythrocyte content) and plasma and not in leucocytes, platelets (or) Stroma indicating that they may bind with haemoglobin.

The reduction in erythrocyte count haematocrit and haemoglobin of Catla catla in the present study can be attributed to the following factors, haemodihition of blood due to the damage of fish organs (Sweilum, 2006), and the haematological Parameters haematocrit, RBC and Hb whose changes can be interpreted as a compensatory response that improves the oxygen carrying capacity to maintain the gas transfer, also indicates a change in the water blood barrier for gas exchange in gill lamellae (Jee et al., 2005).

Erythrocyte level has found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots (Drastichova et al., 2004).

Inhibition of erythropoiesis and an increase in the rate of erythrocyte destruction in haemopoeitic organs are the cause of decrease in RBC count Joshi and Deep (2002) found a significant decrease of RBC count, Hb and haematocrit in endosulfan exposed fish species and indicated the toxic effects of endosulfan on the spleen, liver and anterior kidney.

The decrease in the haemoglobin content in the present study resulting from rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical
brought about by the toxic stress of Lihocin. It is increasingly recognized that toxic substance which is capable of undergoing redox cycling can exert toxic effects via generation of oxygen free radicals. Matkovich and wytans (1981) observed that in cyprinus carpio a quick decrease of haemoglobin content in response to paraquat toxicity.

The haematocrit values decreased when a fish loses its appetite, (or) it is diseased or poisoned by pesticides (Gill and pant, 1985). The reduction in the haematocrit values indicates that the fish suffers from anemia or haemodilution. In addition an alteration in the fish metabolism would also lead to decrease values of haematocrit in Catla catla (Srivastava and Misra, 1979).

Similarly to the present study a significant decrease in the Red cell count was reported in fingerlings of cyprinus carpio exposed to different concentrations from 0.26 to 0.78µg / litre of endosulfan (Chandra Sekhar and Jaya Balan, 1993).

The haematocrit is a measure of how much space red blood cells occupy in blood. It finds significant utility in evaluation of whether the organism is suffering from anemia or not. Decline in Hct content of Catla catla present author infers can seemingly be attributed to the release of erythroblasts (immature erythrocytes) and lysis of erythrocytes in the general circulation which become apparent from day 1 of the experiment (Figure - 3.4). The prevalence of erythroblasts and the extent of damage to RBC by lysis get aggravated as the chronicity of Lihocin progresses. In this context, observations of Srivastava and Mishra (1979) who behold hemolytic anemia due to lysis of erythrocytes in Colisa fasciatus after exposure to lead (Pb) very emphatically support the presently held viewpoint. From the above discussion and observations, it can be safely stated that the insecticide Lihocin by interfering with the normal physiology of RBC possibly result in shrinkage of cell size of RBC which ultimately affect the Hct/PCV of the fish.

In the fish exposed to Silver nanoparticles showed the opposite trend of results obtained against to the fish treated with Lihocin and A. veronii. The results have been showing that RBC count, Haemaglobin and PCV are gradually increased and the animal tried to reach normal level to sustain in the Lihocin intoxication and Aeromonas veronii infected environment.