4.1 General haematological profile of *Garra gotyla gotyla* (Table 2 and Figure 7)

Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal range of various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao, 1986 and Xiaoyun *et al.*, 2009). The analysis of blood indices have proven to be a valuable approach for analyzing the reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al.*, 2001).

It is well known that blood constitutes 1.3-7% of the total body weight of fish and represents one of the most active components which accompanied by haemopoietic organs contribute in metabolic processes by ensuring gas exchange between the organism
and environment. Blood of *Garra gotyla gotyla* like other vertebrates consist of plasma and cellular components. Plasma comprises of 97% water, dissolved salts, electrolytes and hormones. The cellular components include erythrocytes (RBCs), leucocytes (WBC) and thrombocytes. Similar to other hill stream fishes, *Garra gotyla gotyla* also exhibit a normal range of its cellular components. Presently, the haematological parameters of *Garra gotyla gotyla*, fish of present studies in their natural environment (Jhajjar stream) for a period of two years has been studied and a general account have been detailed which include all cellular components.

**Erythrocytes:** Erythrocytes or red blood cells (RBCs) are the most frequently observed formed elements in the blood of *Garra gotyla gotyla*. Mature erythrocytes are elliptical in shape. Cytoplasm of RBC is light violet in colour with a centrally placed nucleus (Figure 7). Cytoplasm of erythrocyte is a site for the deposition of haemoglobin, an important respiratory pigment. These erythrocytes play an important role in the exchange of gases (O₂ and CO₂) between blood and tissues. The total erythrocyte count ranges from 2.2-3.04×10⁶/cmm (Table 2). Cell size has been found to range from 0.065-0.0881mm. Any deviation in form and number of RBCs leads to various pathological conditions in fish under the effect of different stressors (natural and anthropogenic). Increase in the number of erythrocytes is known as polycythemia whereas decrease in their leads to erythrocytopenia.

**Haemoglobin:** Haemoglobin is purple coloured, iron containing respiratory pigment present in the cytoplasm of erythrocytes. It is a conjugated protein formed of four heme groups and a protein called globin. Each heme molecule contains an iron porphyrin ring. Haemoglobin serves to transport oxygen from gills to different tissues of the fish. They carry oxygen in the form of oxyhaemoglobin and carbon dioxide in the form of carboxyhaemoglobin. The Hb content of *Garra gotyla gotyla* during the present study has been found to range from 7.4%-9.5% (Table 2). Decline in Hb content with or without an absolute decrease in RBCs lead to anaemia like condition where upon fish may suffer from hypoxia due to lack of respiratory pigment.

**Haematocrit:** Haematocrit (Hct), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF) is used to express the volume of RBCs in 100ml of whole blood. 38.4% to 44.2% is the range of normal Hct in fish *Garra gotyla gotyla*.
during present study (Table 2). Hct is also used as an indicator of health status of fish. Alterations in Hct values from their normal value can result in various pathological conditions in fish. Increased level of Hct may result in polycythemia and lowered Hct value can imply significant haemorrhages in the fishes.

**Calculated Values**

**Mean corpuscular volume (MCV):** MCV is the measure of average volume of RBCs in whole blood. It is used as an index of the size of RBCs and reflects an abnormal or normal cell division during erythropoiesis. Its increase indicates swelling (macrocytosis) of RBCs and its decrease depicts their shrinkage (microcytosis). It is also an indicative of anaemic condition in fishes i.e. microcytic anemia, normocytic anemia and macrocytic anemia. During microcytic anaemia, MCV ranges below normal range, during normocytic anaemia, MCV though exhibit normal range but Hb remains low and in case of macrocytic anaemia, MCV ranges above normal range. Presently MCV has been found to range from 141.1fl to191.0fl under natural conditions (Table 2).

**Mean corpuscular haemoglobin (MCH):** MCH represent the average haemoglobin content in a red blood cell. The macrocytic RBCs generally have higher MCH values compared to microcytic which usually have lower MCH values. The MCH has been observed to range from 30.7pg to 36.4pg in fish *Garra gotyla gotyla* (Table 2).

**Mean corpuscular haemoglobin concentration (MCHC):** MCHC is also one of important calculated indices of RBCs that has been used to measure average concentration of haemoglobin in a given volume of blood. MCHC is found to be low (hypochromic) in macrocytic anemia but remain normal (normochromic) in macrocytic anemia (due to large size, though the Hb is high but concentration remains normal). The normal range of MCHC has been found to range between 19.0% to 21.5% (Table 2).

**Leucocytes:**

Leucocytes or WBCs by acting as first line of defense against any type of infection or pathogen make an organism immune to fight any kind of possible stressor be it natural or anthropogenic. TLC represents total number of leucocytes per cubic millimeter. Presently normal range of leucocytes lies between $14.25 \times 10^3$/cmm to
17.25×10^3/cmm in fish *Garra gotyla gotyla* (Table 2). The leucocyte count is of diagnostic value in many fish diseases and they respond to any infection by increasing or decreasing its number. Increment in leucocyte number from normal range is called leucocytosis while their decline is known as leucopenia. Leucocytes are further categorized into two categories on the basis of presence or absence of cytoplasmic granules.

1) Agranulocytes 2) Granulocytes

**Agranulocytes:** These are the leucocytes which have rounded or oblong nucleus. They are further categorized into two types.

1) **Lymphocytes:** These are the most abundant cells and constitute 37-53% of the total leucocytes in *Garra gotyla gotyla* (Table 1). Lymphocytes have large and round nucleus with the cytoplasm restricted to thin peripheral layer (Figure 7). Size of lymphocytes range between 0.039-0.052mm in *Garra gotyla gotyla*. Lymphocytes are the sites for antibody production and hence provide protection against any kind of antigen and pathogen. Lymphocytosis is increase in their number and lymphocytopenia is represents the decline in the number of lymphocytes from their normal values. Any deviation, increase or decrease from normal value is indicative of infection.

**Monocytes:** Monocytes are the largest size among all the leucocytes and contribute approximately 2-5% of total leucocytic population in fish *Garra gotyla gotyla* (Table 2). The size of monocyte ranges from 0.082-0.187mm. These have kidney shaped nucleus which is placed eccentrically in the cytoplasm (Figure 7). Monocytes represent the second line of defense after lymphocytes and play an important role in phagocytosis, being phagocytic in nature. They engulf the damaged cells as well as bacteria. Monocytosis (increase in number of monocytes) and monocytopenia (decrease in number of monocytes) are the apparent pathological symptoms observed under any kind of stress.

**Granulocytes:** These are the leucocytes which have granular cytoplasm and lobed nucleus. They are further categorized into three types.1) neutrophils, 2) eosinophils and 3) basophils.
**Neutrophils:** Neutrophils contribute approximately 16-30 % of the total number of leucocytes (Table 2). The characteristic feature of neutrophil is the presence of lobed nucleus (2-3 lobed) (Figure 7). Besides monocytes, these are the chief phagocytic cells in *Garra gotyla gotyla*. They are also known as soldiers or the scavengers of the body.

**Eosinophils:** Eosinophils are oblong in shape with granulated cytoplasm (Figure 7). They constitute about 3% of total leucocytic population in *Garra gotyla gotyla*. They also play an important role in process of phagocytosis. They are known to contain histamines (Clark *et al.*, 1975) and thus may play role against any kind of allergies. Mast cell activation has also been reported in eosinophils by Pilipensky *et al.* (2002). The size of eosinophils has been observed to range between 0.063-0.078mm.

**Basophils:** Basophils represent the least abundant class of granulocytes. They are recognized by their large deep bluish purple cytoplasmic granules overlying the nucleus and constitute only about 2% of total leucocytic population in fish *Garra gotyla gotyla* (Figure 7 and Table 2). Their size ranges from 0.065-0.080mm. They are also phagocytic in nature.

**Thrombocytes:** They are round or oval in shape (Figure 7) and their number ranges from 8% to 42 % in fish *Garra gotyla gotyla* (Table 2). They represent the second largest population of leucocytes after lymphocytes. Their cytoplasm is reddish or purplish in colour. Thrombocytes are multifunctional cells involved in blood coagulation (Witeska, 2005), eicosanoids releasing (Hill *et al.*, 1999) and immune defense (Tavarse-Dias and Moraes, 2004). They also represent a link between innate and adaptive immunity. Size of thrombocytes range from 0.054-0.068mm in *Garra gotyla gotyla*.

From above discussion on the general profile of haematological parameters of fish *Garra gotyla gotyla*, it can be concluded that fish *Garra gotyla gotyla* like other hill stream fishes exhibit a specific range of its haematological parameters and any deviation from these normal values reflect a state of stress under the effect of natural or anthropogenic stress.