Observations and Results

4.1. Head kidney of healthy *Cirrhinus mrigala*

4.1.1. Morphological studies

The kidney of *Cirrhinus mrigala* is divided into two distinct parts, an anterior part, the head kidney and a posterior part, the trunk kidney. The two lobes of the head kidney are leaf like and are joined by a bulbous portion to the trunk kidney. Kidney is situated dorsal to the abdominal cavity (Fig. 7).

4.1.2. Histological studies

Routine histological studies under light microscope showed that the head kidney was covered by a thin strand of collagen fibres. It was divisible into two distinct regions, outer cortex and inner medulla. Renal tubules were scanty (Fig. 8).

Histological sections showed presence of erythroid and lymphoid cells in both cortex and medulla of the kidney. The presence of more lymphoid cells and less erythroid cells have been found in the cortex compared to medulla.

4.1.3. Ultra microscopic studies

Cells present in the head kidney were varying numbers of lymphomyeloid and erythroid cells and they are not tightly packed Adjacent to blood capillaries or blood sinusoids lymphoblasts were predominantly found along with granulocytes (melanomacrophages), monocytes, plasma cells and epithelial cells (Fig. 9). Other regions showed presence of heterogenous population of cells consisting of neutrophils, neutrophilic myelocytes, haemoblasts, mature erythrocytes, thrombocytes, erythroblasts, lymphocytes and lymphoblasts etc (Fig. 10).

Occasionally presence of epithelial cells forming a network with a few lymphocytes, thrombocytes was detected. Cystic cavity with pyknotic cells was also found (Fig. 11).

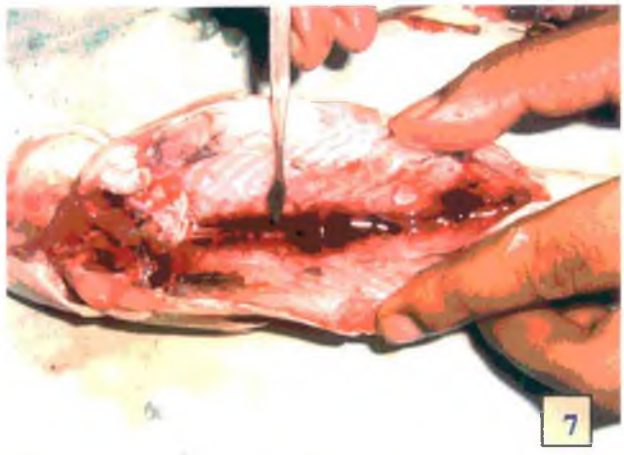
Fig. 7: Head kidney of a healthy *Cirrhinus mrigala.*

Fig. 8: Histological section of head kidney of a healthy *C. mrigala* showing lymphoid and erythroid cells alongwith regional distinction as cortex and medulla.

Fig. 9: Electron microscopic picture (TEM) of head kidney of healthy *C. mrigala* showing different ells, small lymphoid haemoblast (shl), lymphocyte (lym), monocyte, melano macrophages and epithelial cells.

Fig. 10: Electron microscopic picture (TEM) of head kidney of healthy *C. mrigala* showing different cells, neutrophils (N), neutrophilic myelocytes (NM), haemoblasts (Hb), mature erythrocytes (ME), thrombocytes (Th), erythroblasts (EB), lymphocytes (L) and lymphoblasts (LB).

Fig. 11: Electron microscopic picture of head kidney of healthy *C. mrigala* showing the cellular composition predominantly with epithelial cells, forming a network with a few lymphocytes, thrombocytes and cystic cavity with pyknotic cells.



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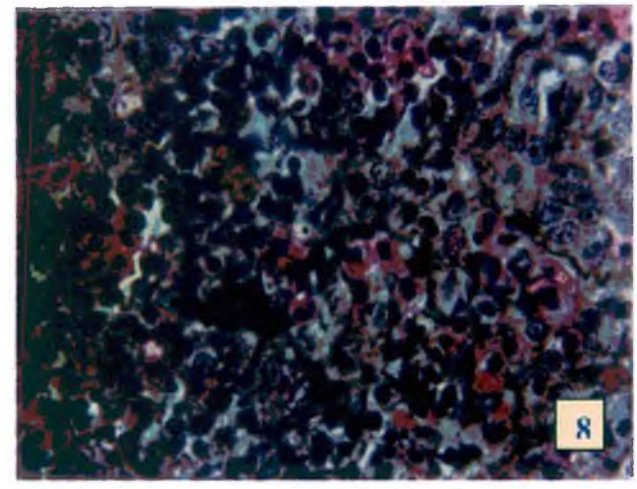

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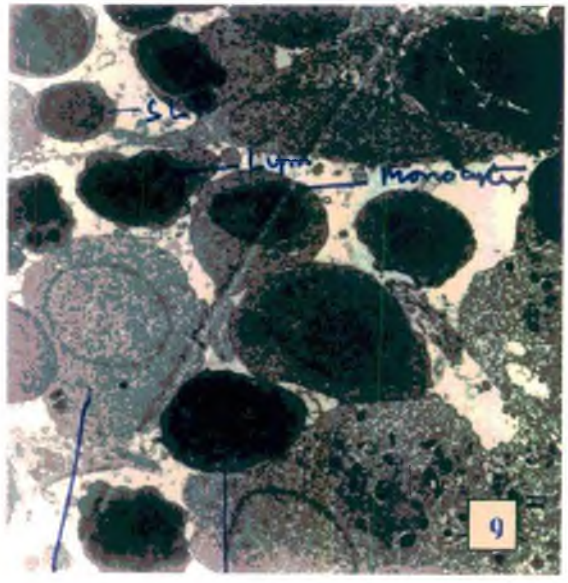

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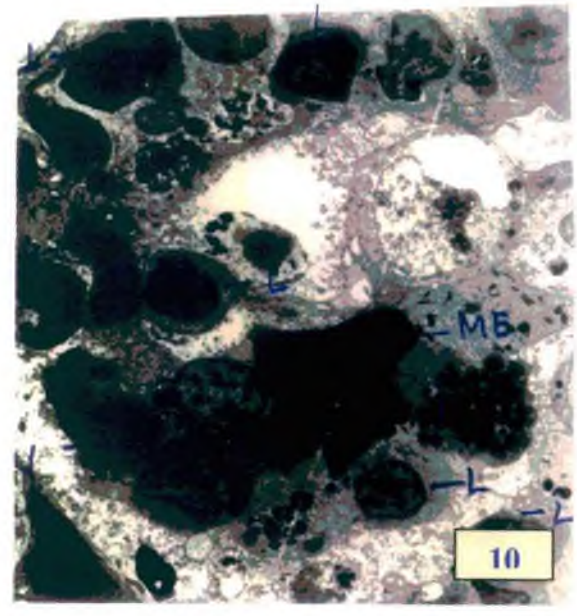

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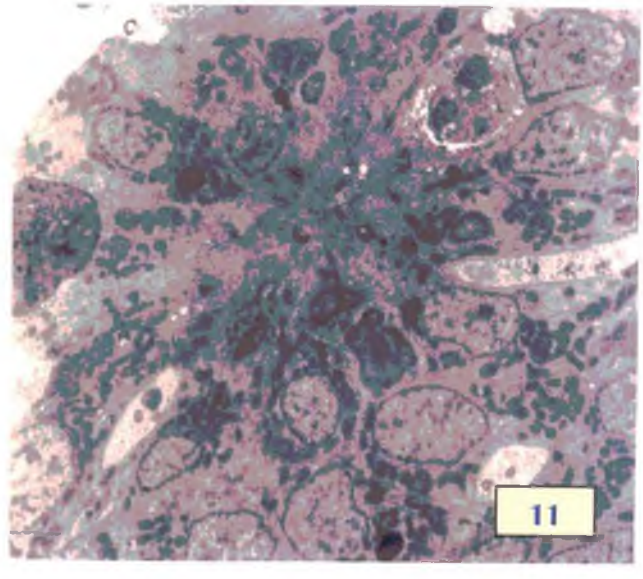

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4.2. Spleen of healthy *Cirrhinus mrigala*

4.2.1. Morphological studies

Spleen of *C. mrigala,* is an irregular structure and dispersed in form. It is located on or between the loops of intestine.

4.2.2. Histological studies

Histological studies of spleen under light microscope showed presence of both red pulp and white pulp regions. White pulp was surrounded by the red pulp. The red pulp was composed of different developmental stages of erythrocytes with a few lymphocytes while the white pulp region was comprised of reticular cells (Fig. 12).

4.2.3. Ultra structural studies

In the spleen the cells were more tightly packed. The cell types found in the spleen were of following types: thrombocytes with electro lucent cytoplasm, lymphocytes, reticular cells, monocytes with prominent cytoplasmic organelles (Fig 13) and neutrophils along with type-I and type-II granules (Figs. 14, 15, 16, 17, 18 and 19).

Presence of cords of erythrocytes was recorded in some regions. The cords were separated by connective tissue and electron lucent reticular cells.

4.3. Thymus of healthy *Cirrhinus mrigala*

4.3.1. Morphological studies

Thymus is a paired organ which is reddish in colour, triangular in shape and occupies the dorsolateral regions of the opercular cavity (Fig. 20).

Fig. 12: Histological section of spleen of healthy *C. mrigala* showing red pulp (rp) and white pulp (wp) regions.

Fig. 13: E/M (TEM) picture of spleen of healthy *C. mrigala* showing different cell types like Young reticulocyte, Mature erythrocyte (ME), Monocyte (M), Neutrophilic myelocyte (NM), Reticulo erythrocyte (RE) etc.

Fig. 14: E/M (TEM) picture of spleen of healthy *C. mrigala* showing a typical thrombocyte electro-lucent cytoplasm and extensive surface connected canalicular system of cytoplasm.

Fig. 15: E/M (TEM) shows the picture of a typical splenic lymphocyte.


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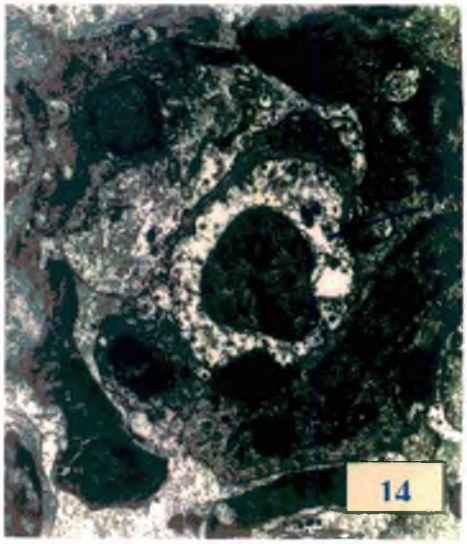
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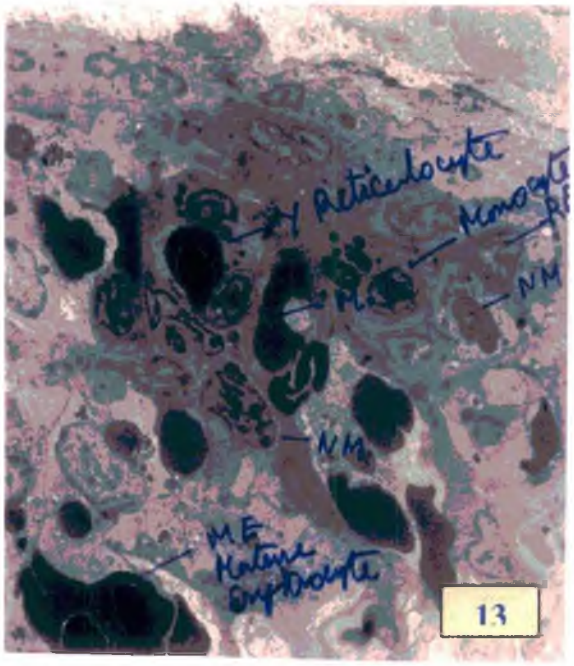
Reticulocyte
NM
NM
M.E
Mature
Erythroblast
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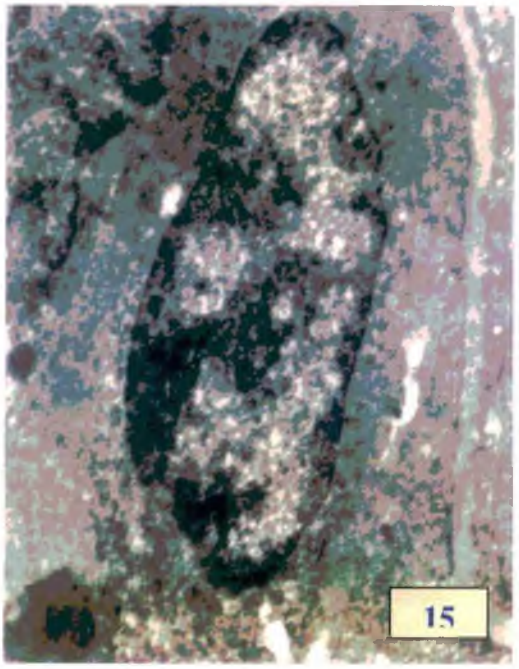
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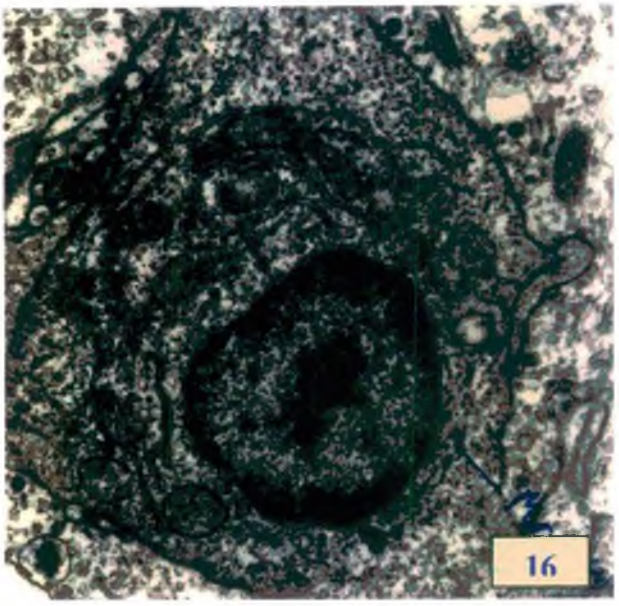
Fig. 16: E/M (TEM) shows the picture of typical cell found in spleen of healthy *C. mrigala.*

Fig. 17: E/M (TEM) shows a neutrophil present in spleen of healthy *C. mrigala* with Type-I and Type-II granules.

Fig. 18: E/M (TEM) shows a typical cell surrounded by red blood cells in spleen of healthy *C. mrigala.*

Fig. 19: E/M (TEM) shows the cords of erythrocytes along with indistinct RT or Reticulo endothelial cells in spleen of healthy *C. mrigala.*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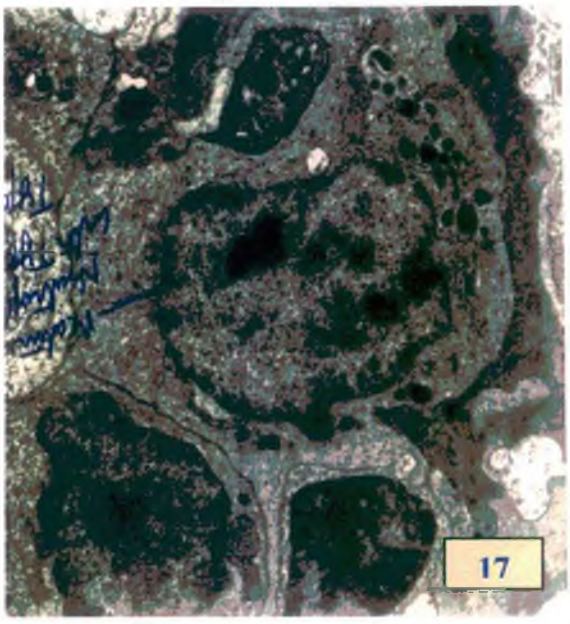
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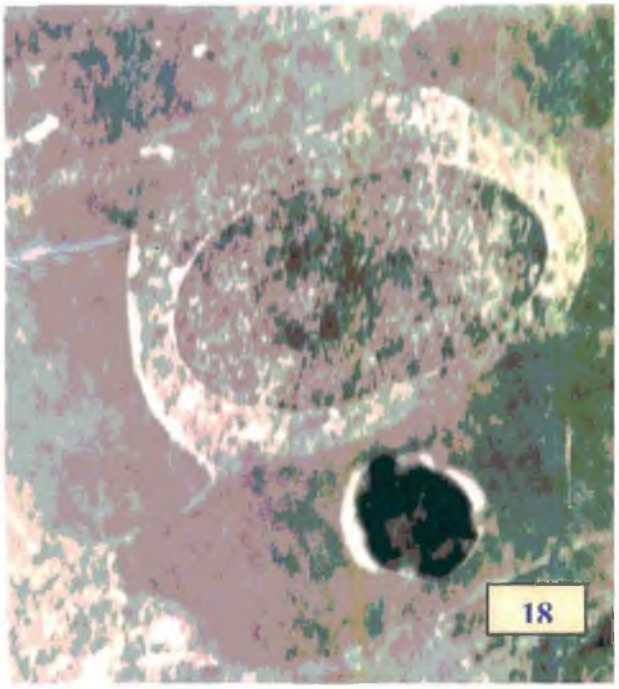
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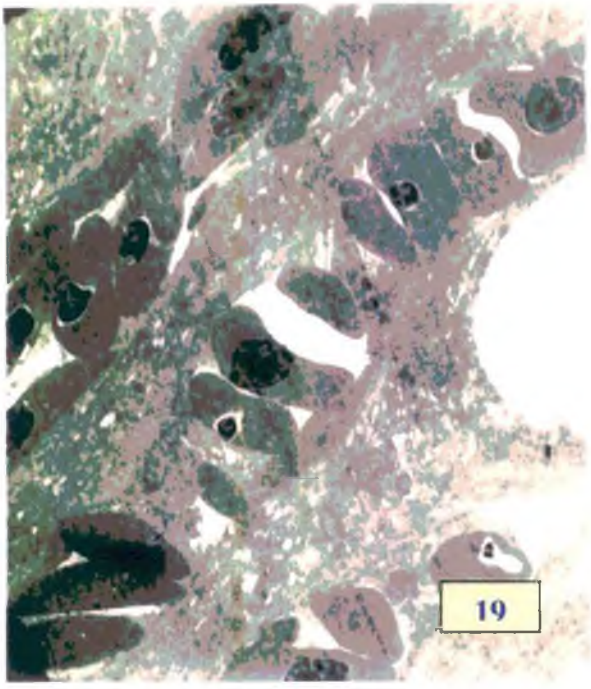
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Table2. The size and weight of the thymus of healthy *C. mrigala*

Weight of fish (gm)	Thymus		
	Length (mm)	Width (mm)	Weight (gm)
72.00±5.40	3.928±0.2177	2.815±0.2404	0.0116±0.00245

Mean±S.D; n=10

The weight of thymus of healthy *C. mrigala* weighing 72.00±5.40gm was 0.0116±0.00245 gm (Table 2). The Fig. 37 shows the comparison between the weights of thymus of healthy and EUS affected *C. mrigala.*

The length and breadth of thymus were 3.928±0.2177mm and 2.815±0.2404 mm respectively.

4.3.2. Histological studies

Histological sections of the thymus of healthy *C. mrigala* showed the presence of a thin capsule which sends trabeculae into the stroma to form lobules. The lobules were not completely separated but interconnected due to discontinuous nature of the trabeculae. The stroma was not differentiated into cortex and medulla. The stroma showed presence of hemopoietic cells arranged in cords (Fig. 21). The cords of hemopoietic cells contained mainly developing blood cells. Reticular epithelial cells were present in the space between the cords of hemopoietic cells.

Hassall's corpuscles like structures consisting of concentric layer of epithelial reticular cells were also detected.

4.3.3. Ultra microscopic studies

Transmission electron microscopic studies showed the presence of cords of erythrocytes separated by cords of cells with secretory granules. Apart from cords of

erythrocytes and cords of cells with secretory granules, a considerable number of lymphocytes, heterogenous epithelial cells, macrophages, pigment cells (melano macrophages), granulocytes, myoid cells were also detected (Figs. 22, 23 and 24).

Heterogenous epithelial cells community mainly constituted four types of epithelial cells: reticular epithelial cells (RT), epithelial cells with semi electron dense secretory granules, large epithelial cells with cystic cavity and limiting epithelial cells along with capsule (Fig. 22).

Reticular epithelial cells were characterized by the large euchromatic nucleus and with electron lucent cytoplasm. Epithelial cells with semi electron dense secretory granules were frequent in between two cords of erythroid cells. Large epithelial cells with cystic cavities were found less frequently in an inner part of thymus. Limiting epithelial cells along with connecting tissue capsule were also found (Fig. 25).

Pigment cells or melano macrophages characterized by a huge number of electron dense granules in the cytoplasm were also detected (Fig. 26). Mature myoid cells mainly occupied the inner region of thymus and were characterized by large round or oval cells with electron dense nucleus and cytoplasm. These cells were mostly found in association with smooth muscles (Fig. 27).

4.4. Head kidney of EUS affected *Cirrhinus mrigala*

4.4.1. Morphological studies

Morphological study of the head kidney of EUS affected *C. mrigala* showed no significant structural changes. Only the colour of head kidney of EUS affected *C. mrigala* appeared pale red compared to the head kidney of healthy fishes.

4.4.2. Histological studies

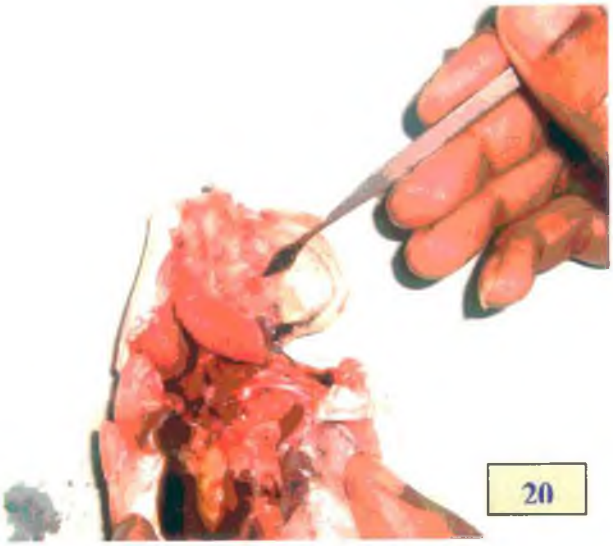
Light microscopic studies of the head kidney of naturally infected *C. mrigala* revealed significant changes in the histological structure of the head kidney.

Fig. 20: Picture shows the position of thymus in the fish.

Fig. 21: Histological section of thymus of healthy *Cirrhinus mrigala* showing inter connected lobules, trabeculae, cords of erythrocytes separated by other cell types.

Fig. 22: E/M (TEM) picture showing the cords of erythrocytes separated by other cell types present in thymus of healthy *C. mrigala.*

Fig. 23: E/M (TEM) picture of thymus of healthy *C. mrigala* showing different cells like, mature erythrocyte (MR), cells with huge secretory granules, Hypertrophied epitheliam (H.ep) etc.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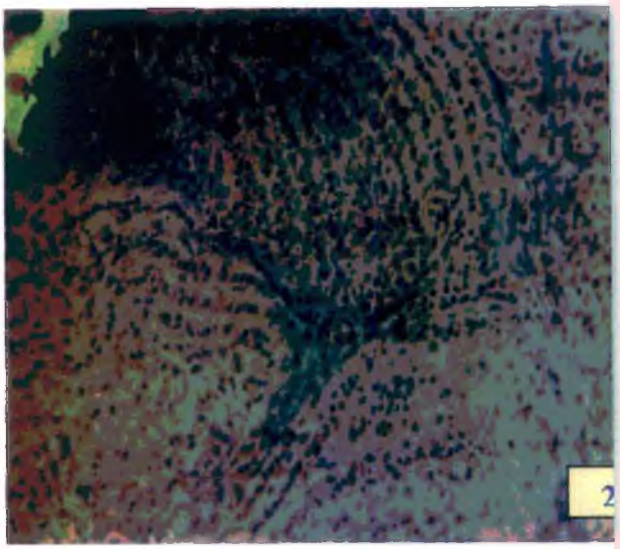
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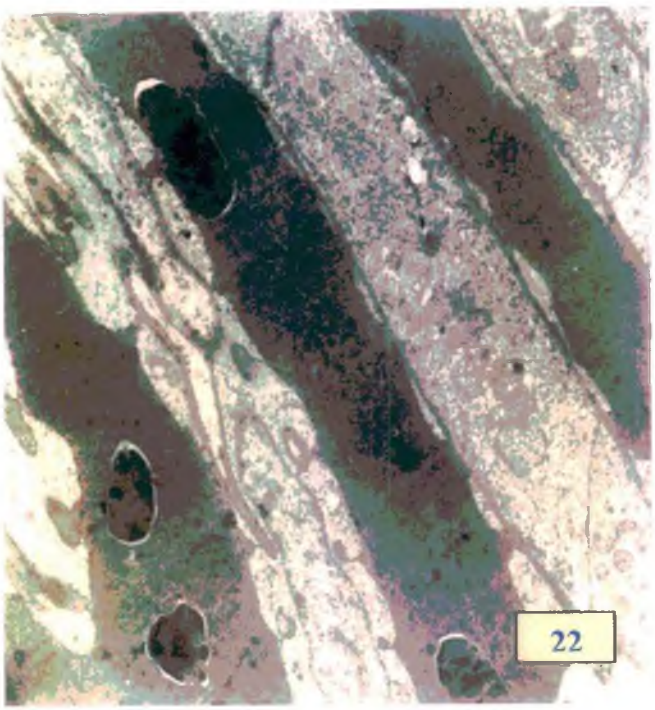
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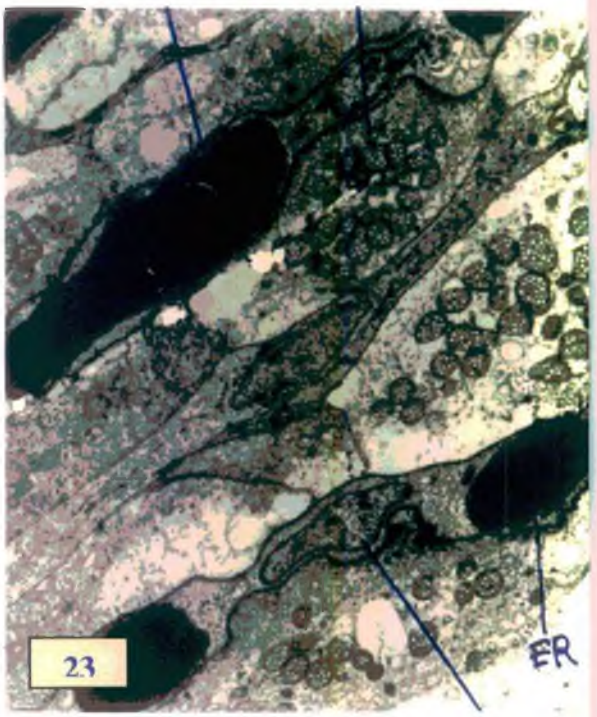
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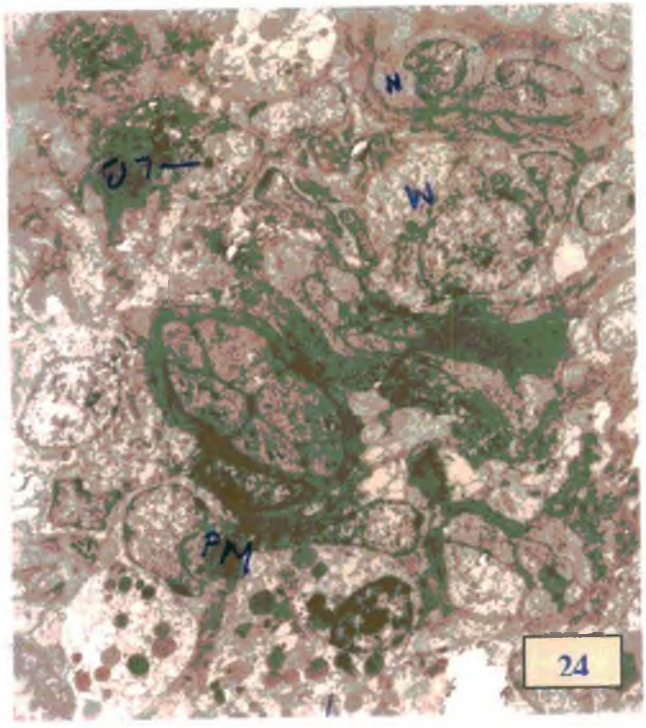
Fig. 24: Picture shows number of lymphoid cells within a network of epithelial cells.

Fig. 25: E/M (TEM) picture of thymus of healthy *C. mrigala* showing different cells like, lymphocytes, heterogeneous epithelial cells, macrophages, pigment cells (melano macrophages), granulocytes and myeloid cells etc.

Fig. 26: E/M (TEM) picture of thymus of healthy *C. mrigala* shows a cell with huge number of electron-dense granules.

Fig. 27: E/M (TEM) of thymus of healthy *C. mrigala* shows a typical myoid cell.



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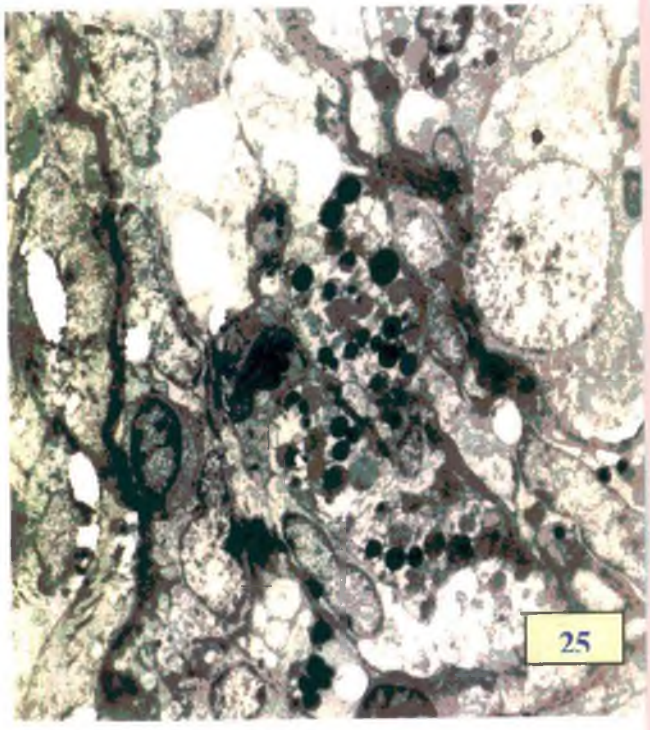
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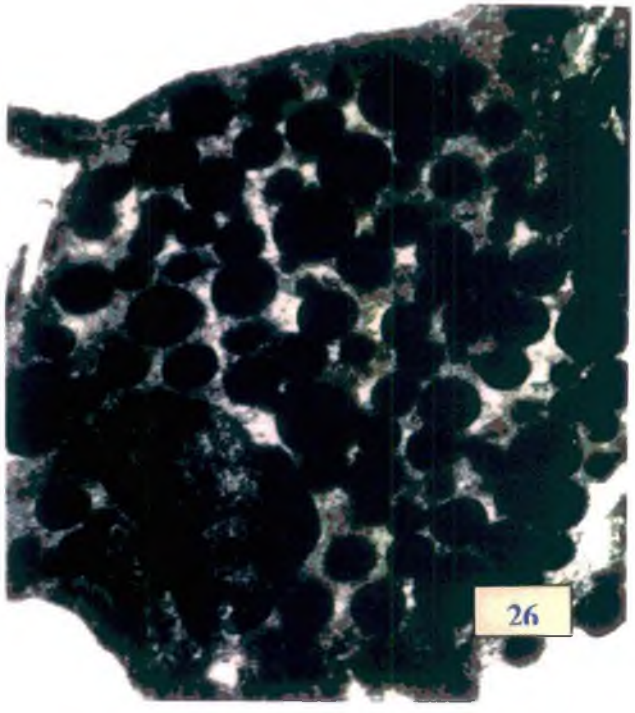
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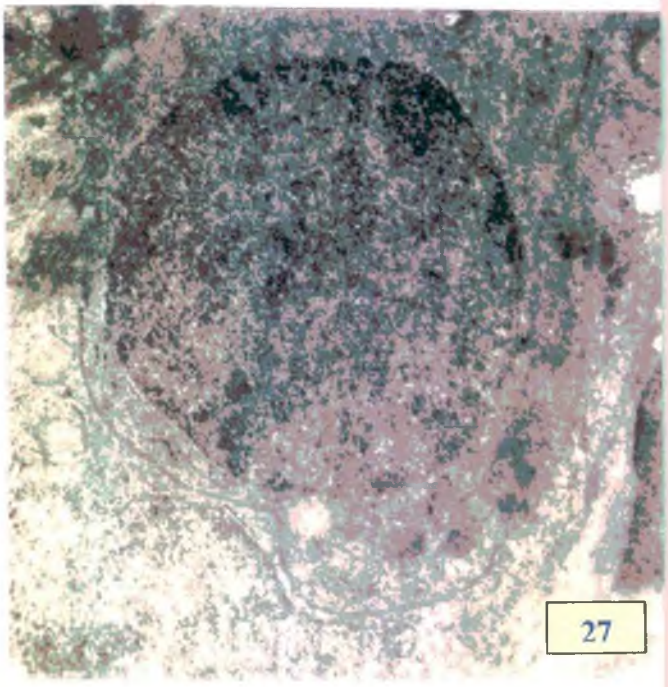
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Haemorrhages in some areas were detected along with the presence of haemosiderin laden macrophages. Necrotic changes were also very common in the head kidney of naturally infected *C. mrigala* (Figs. 28 and 29).

4.4.3. Ultra microscopic studies

Ultra structural studies of head kidney of EUS affected *C. mrigala* showed a considerable change. Presence of bacteria was found throughout the tissue. Along with the presence of bacteria, large areas of renal tissue showed necrotic changes. Melanin depositions and fibrin clumps were also observed (Figs. 30, 31 and 32).

Concentrations of erythrocytes in head kidney were significantly less compared to head kidney of healthy fish.

4.5. Spleen of EUS affected *Cirrhinus mrigala*

4.5.1. Morphological studies

No significant morphological changes of spleen of EUS affected *C. mrigala* were identified.

4.5.2. Histological studies

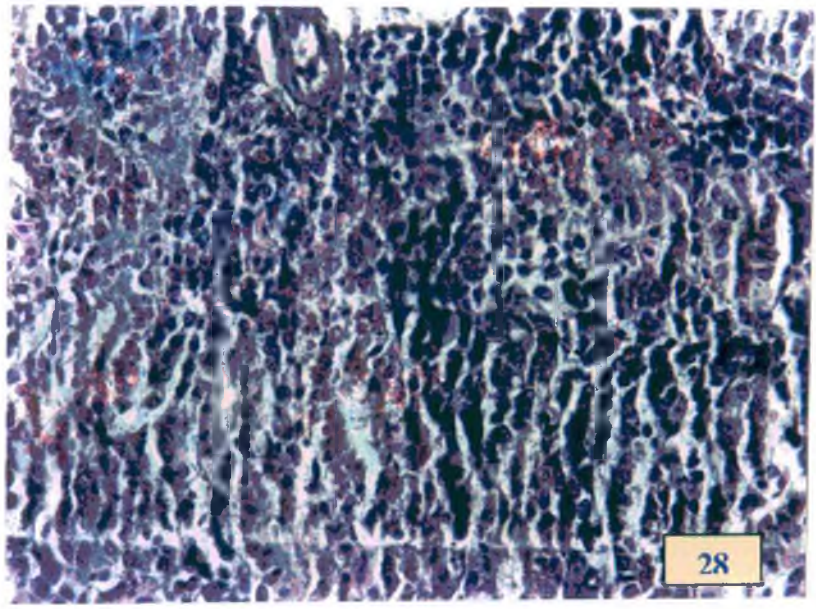
Necrotic changes along with vacuolation in the white pulp regions of spleen of naturally infected *C. mrigala* were noticed. Haemorrhages were also found (Fig. 33).

4.5.3. Ultra microscopic studies

Ultra structural studies of spleen of EUS affected *C. mrigala* showed some pathological changes. Necrotic changes and presence of fibrin clumps, depositions of melanin were noticed. Considerable decrease of erythrocytes was also observed. The splenic tissue of affected fish showed the presence of bacteria. Macrophages laden with bacteria were detected in some areas of the spleen (34, 35 and 36).

Fig. 28: Histological section of head kidney of EUS affected *C. mrigala* shows necrotic changes in the renal tissue.

Fig.29: Histological section of head kidney of EUS affected *C. mrigala* shows necrotic changes in the renal tissue in high power.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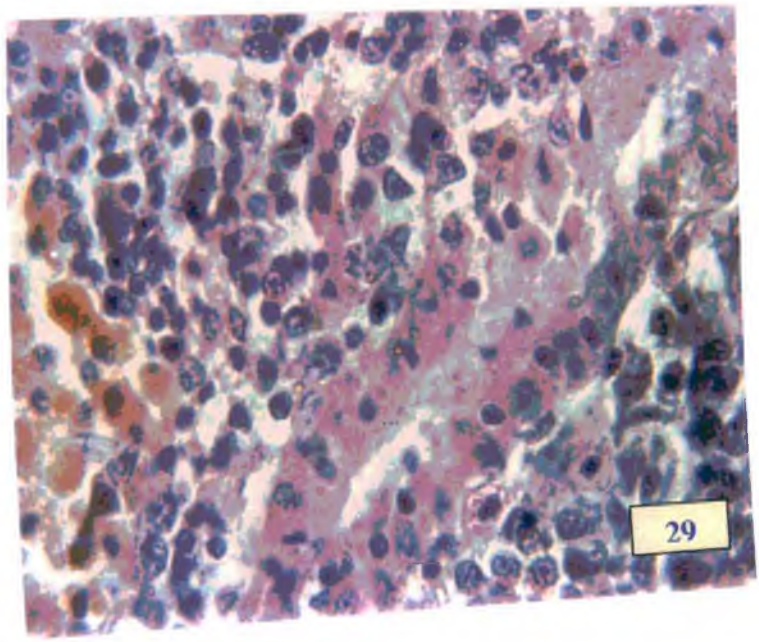
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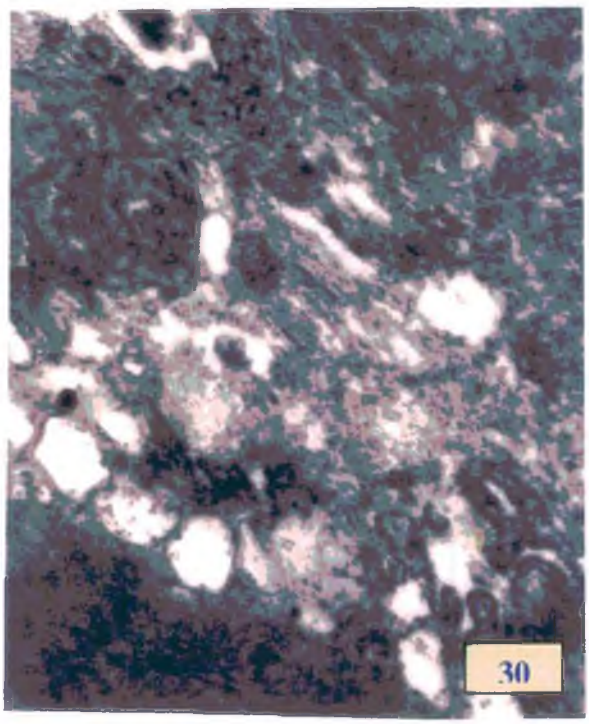
Fig.30: E.M studies of head kidney of EUS affected *C. mrigala* shows the presence of bacteria in renal tissue.

Fig.31: E.M studies of head kidney of EUS affected *C. mrigala* shows the melanin deposition and fibrin clumps around a lymphocyte.

Fig.32: E.M studies of head kidney of EUS affected *C. mrigala* shows the presence of necrotic change in renal tissue.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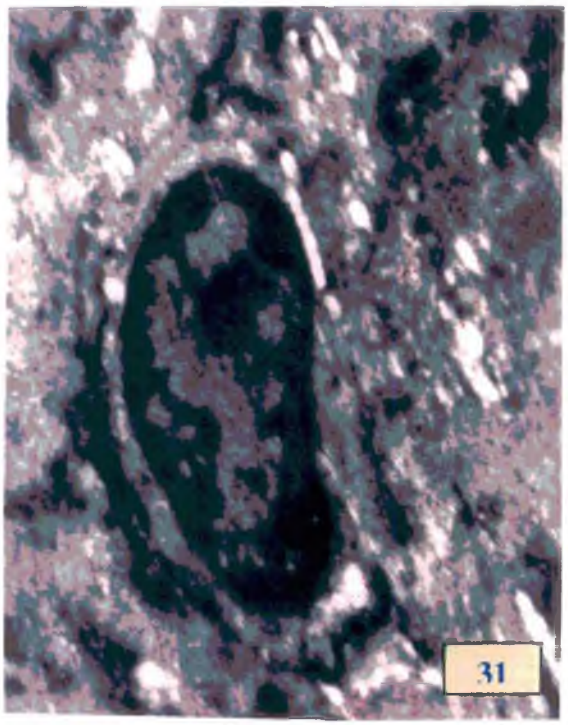
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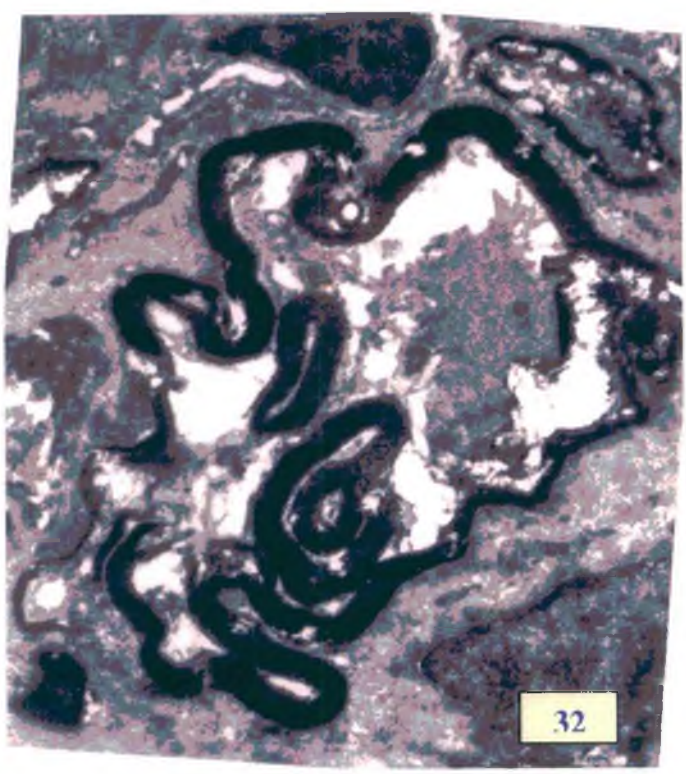
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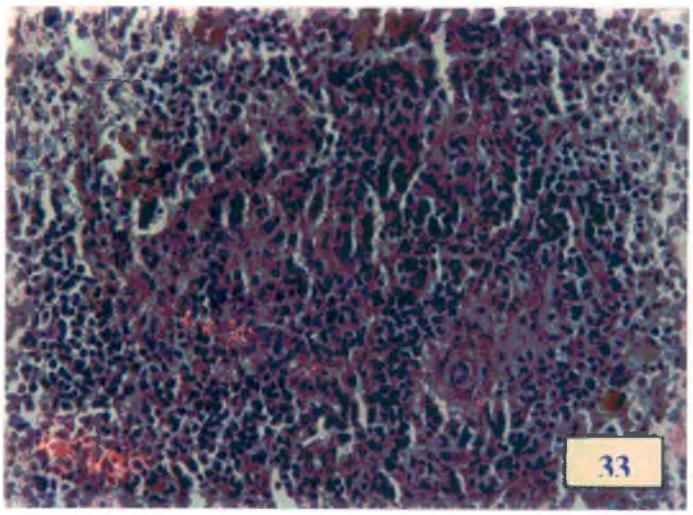
Fig.33: Histological section of spleen of EUS affected *C. mrigala* shows the necrotic changes in splenic tissue.

Fig.34: E.M studies of spleen of EUS affected *C. mrigala* shows the presence of melanin deposition and fibrin clumps.

Fig.35: E.M studies of spleen of EUS affected *C. mrigala* shows the presence of bacteria in between erythroid and lymphoid cells.

Fig.36: E.M studies of spleen of EUS affected *C. mrigala* shows the presence of huge gap between erythrocytes.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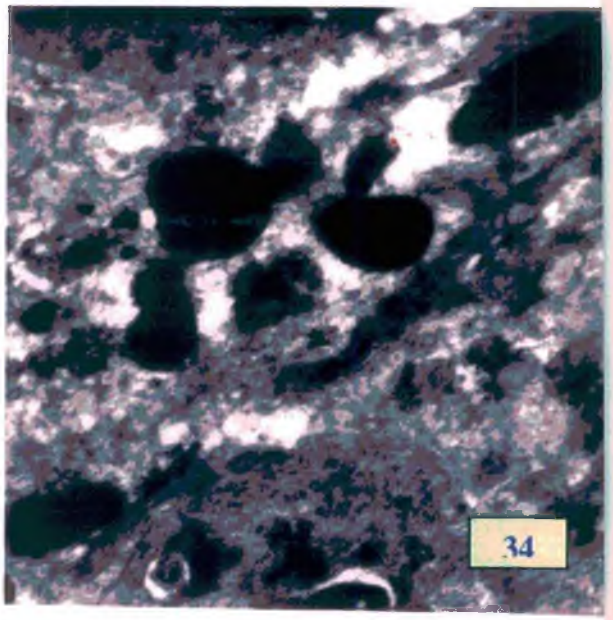
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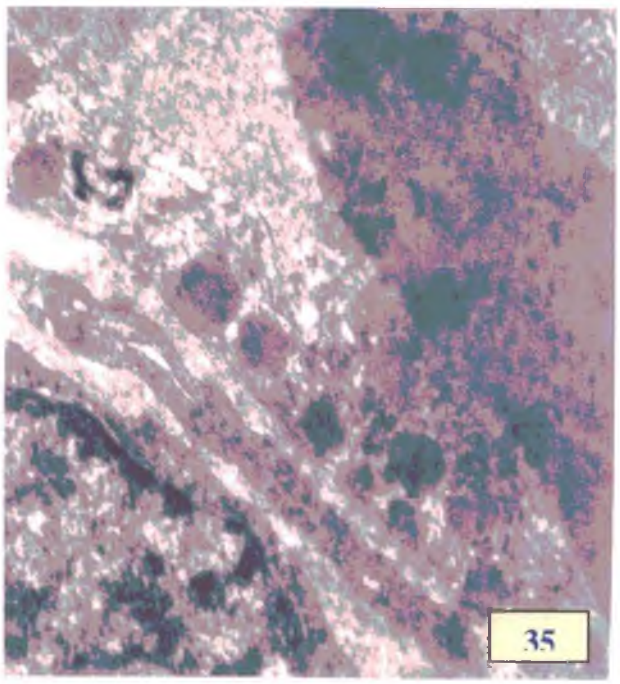
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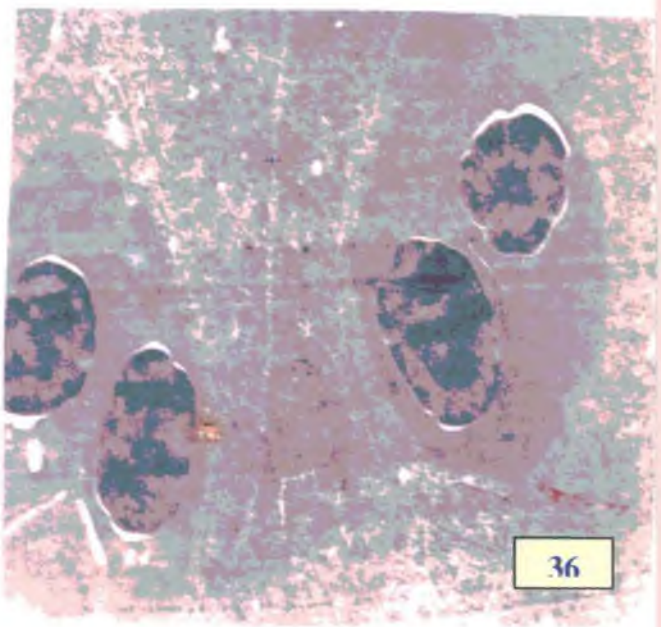
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4.6. Thymus of EUS affected *Cirrhinus mrigala*

4.6.1. Morphological studies

Morphological studies of thymuses of EUS affected *C. mrigala* revealed that thymuses of EUS affected fishes were to some extent enlarged compared to that of the healthy fishes. The colour of thymuses of EUS affected fishes appeared more redish.

The average weight of thymus of EUS affected *C. mrigala* weighing 58±6.7494 gm was 0.01468±0.001349gm and the average length and breadth of thymus were 4.714±0.1646mm and 3.31±0.2671 mm respectively (Table 3). The Fig. 37 shows the comparison between the weights of thymus of healthy and EUS affected *C. mrigala.*

Table3. The size and weight of thymus of EUS affected *C. mrigala*

Weight of fish (gm)	Thymus		
	Length (mm)	Width (mm)	Weight (gm)
58±6.7494	4.714±0.1646	3.31±0.2671	0.01468±0.001349

Mean±S.D; n=10

4.6.2. Histological studies

Histological observations of thymuses of EUS affected fishes showed highly cosinophillic areas near the capsule of the thymus which contained erythroblastic islets, cords of mature erythrocytes and reticular epithelial cells (Figs. 38 and 39). In some areas erythrocytes were replaced by reticular epithelial cells with large nucleolus. Sinuses filled with blood cells were frequently present. Hassall's corpuscles like structures were also detected near the trabeculae in the thymus of EUS affected fishes.

Comparison between the weight of thymus of healthy and EUS affected *C. mrigala*

Fig. 37

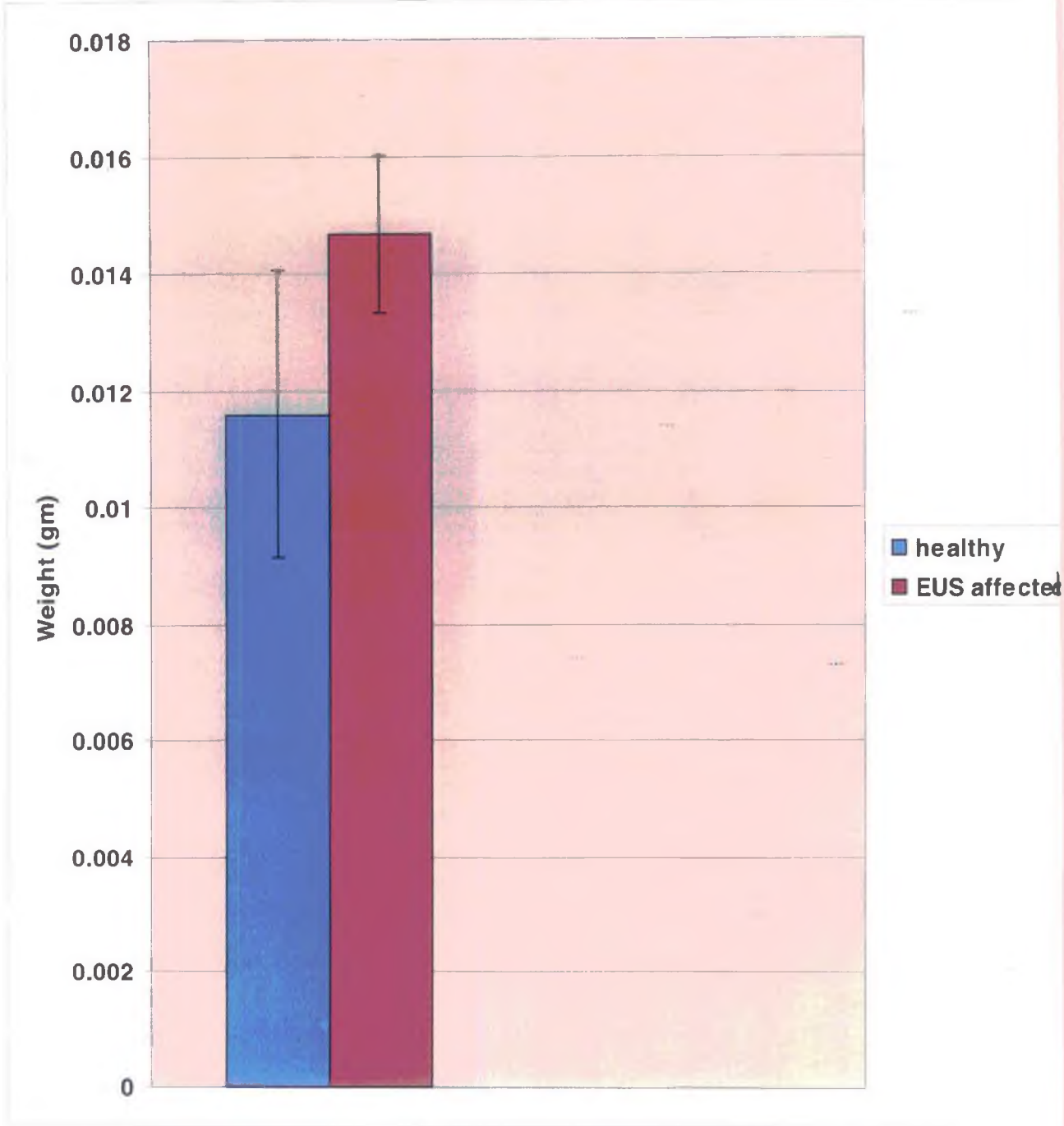
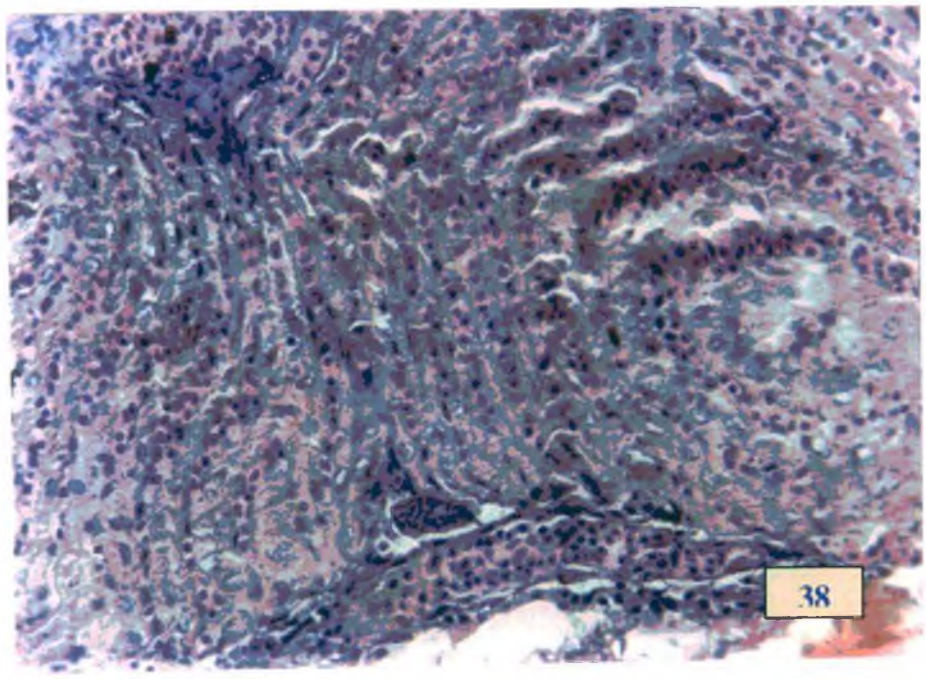


Fig.38: Histological section of thymus of EUS affected *C. mrigala* shows the necrotic changes and presence of erythroid islets.

Fig.39: Histological section of thymus of EUS affected *C. mrigala* shows the necrotic changes along with active macrophages cells in high power.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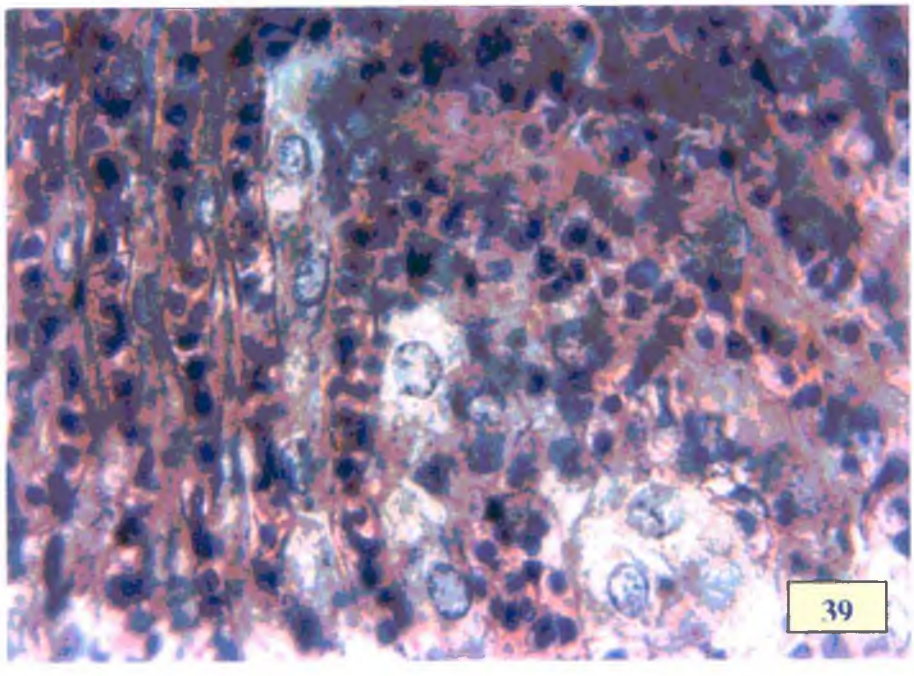
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4.6.3. Ultra microscopic studies

Ultra structure of thymus of EUS affected *C. mrigala* showed the presence of huge number of lymphoid cells which were connected by the desmosomes (Fig. 40). Apart from desmosomes different types of blood cells especially erythroids were also detected but necrotic changes as well as degenerative blood cells were also observed. Bacteria were located extra cellularly and in some cases fibroblasts also appeared to be invaded by bacteria (Fig. 41). Along with the presence of bacteria in the sub capsular and inner zones of thymic parenchyma, necrosis of connective tissue of these regions were also identified. The necrotic changes were established by the disruptions of collagen fibres and of the basal membranes. Pyknotic cells and macrophages with large residuals bodies were also noticed. But pharyngeal epithelium covering thymus showed no significant changes.

4.7. Studies on blood cell profile in healthy *Cirrhinus mrigala*

4.7.1. Morphological and Morphometric studies

Erythrocyte

Erythrocytes of healthy *C. mrigala* under phase contrast microscope appeared either elliptical or oval in form with clearly visible nucleus (Fig. 42). The cytoplasms appeared bluish green when stained with Leishman stain and the nuclei appeared deep magenta in colour. In Sudan Black B stain the cytoplasm of erythrocytes appeared grey in colour and the nuclei appeared dark black. The erythrocytes were PAS negative.

The average cell diameter was 6.0±0.5986 μm while the nuclei diameter was 2.64±0.3375 μm resulting n-c ratio = 1: 2.27.

Leucocytes

Agranulocytes

Lymphocytes

In Leishman stain the lymphocytes appeared almost round in shape. Nucleus took acidophilic stain and appeared deep magenta in colour. The thin rim of cytoplasm encircling the nucleus took basophilic stain and appeared bluish in colour (Figs. 42 and 43).

In small lymphocytes (Fig. 42) the amount of cytoplasm encircling the nucleus are higher in comparison to the cytoplasm present in large lymphocytes (Fig. 43).

The cytoplasm of lymphocytes did not take any stain, and it appeared colourless when stained with Graham-Knoll-benzedine (counterstained with Giemsa). The cytoplasms of lymphocytes showed mild reaction when blood smears were stained with PAS. The nuclei of lymphocytes showed dark colour surrounded by a grey rim of cytoplasm when stained with Sudan Black B (Fig. 44).

The average cell diameter of small lymphocyte was 3.72±0.39 μm while the average nuclei diameter was 2.5±0.64 μm resulting the n-c ratio = 1: 1.48.

Monocytes

The shapes of the nuclei varied from oval to kidney shaped and occupied a peripheral position in the cell.

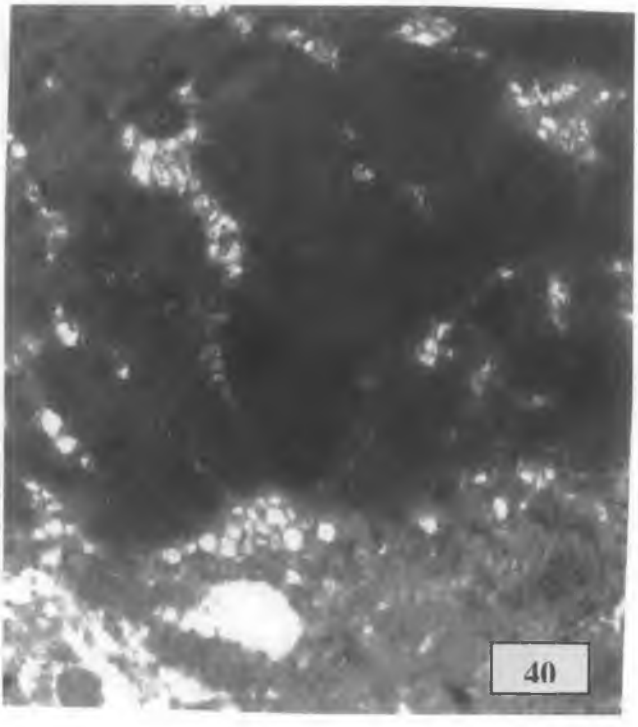
In Leishman stain the nuclei took a very light magenta colour and cytoplasm took a light blue colour. The cytoplasm appeared almost granules free (Fig. 45). The cytoplasms of monocytes showed a slightly stronger reaction for PAS than lymphocytes but like lymphocytes the nuclei of monocytes did not show any PAS reactivity (Fig. 46).

The average diameter of nucleus was 10.33±1.84 μm while the cell diameter was 17.23±2.46 μm resulting the n-c ratio = 1: 1.67.

Granulocytes

Fig.40: E.M studies of thymus of EUS affected *C. mrigala* shows ruptured basement and fibroblast, desmosomes etc.

Fig.41: E.M studies of thymus of EUS affected *C. mrigala* shows the presence of bacteria in thymic tissue.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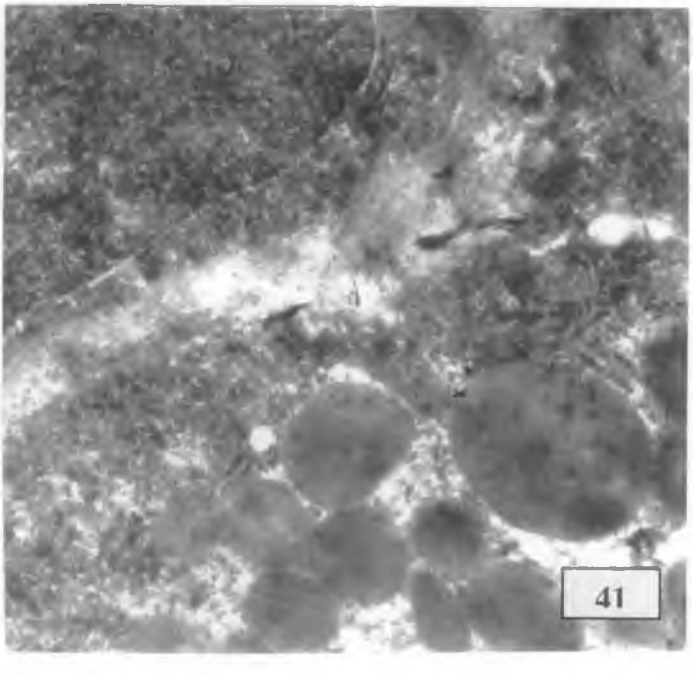
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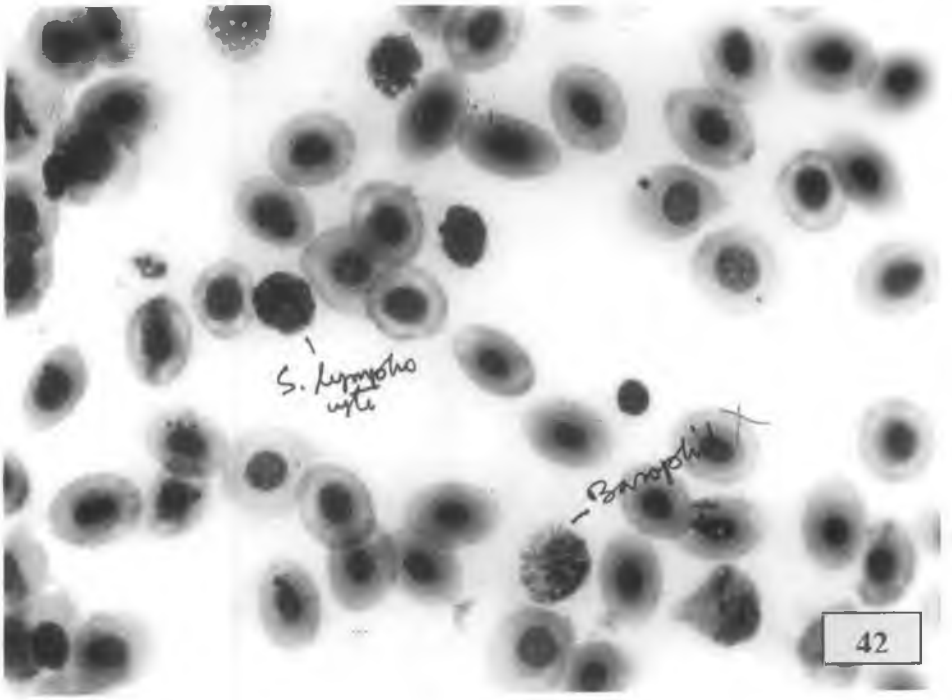
Fig.42: Blood smear of *C. mrigala* stained with Leishman stain shows different blood cells like, erythrocytes (E), small lymphocyte (sl) and basophil (B).

Fig. 43: Blood smear of *C. mrigala* stained with Leishman stain showing the presence of large lymphocyte along with erythrocytes.



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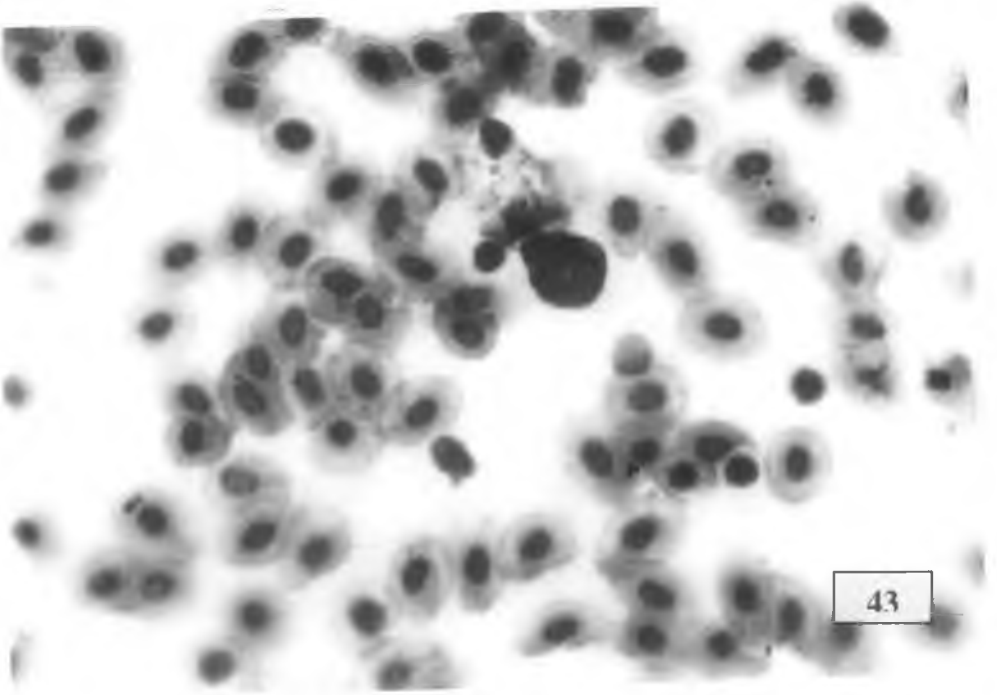

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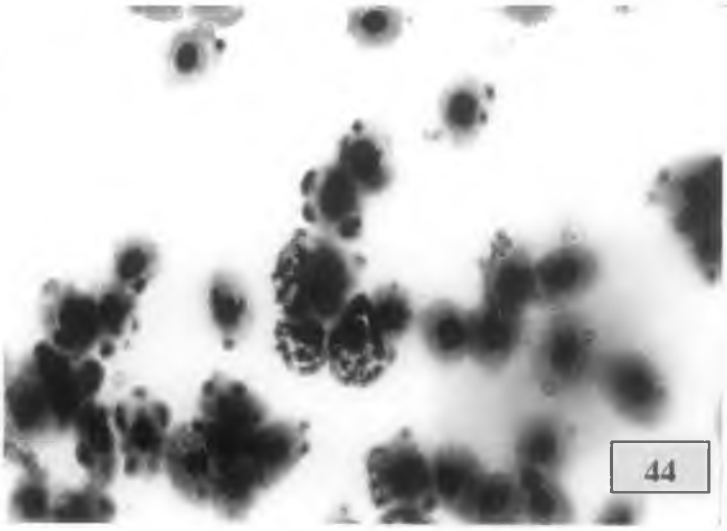
Fig. 44: Blood smear of *C. mrigala* stained with Sudan Black-B showing different blood cells like, erythrocytes, lymphocytes and granulocytes.

Fig.45: Blood smear of *C. mrigala* stained with Leishman stain shows the structure of a typical monocyte and eosinophils.

Fig.46: Blood smear of *C. mrigala* stained with PAS reaction showing the presence of a typical monocyte.

Fig.47: Blood smear of *C. mrigala* stained with Leishman stain shows the presence of a neutrophil.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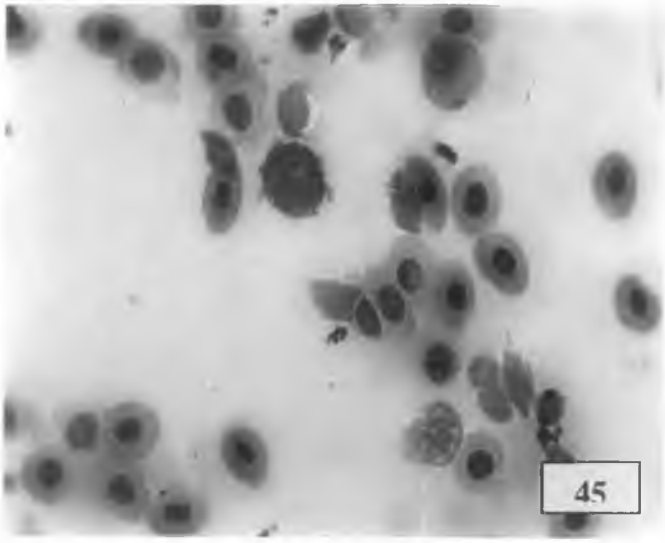
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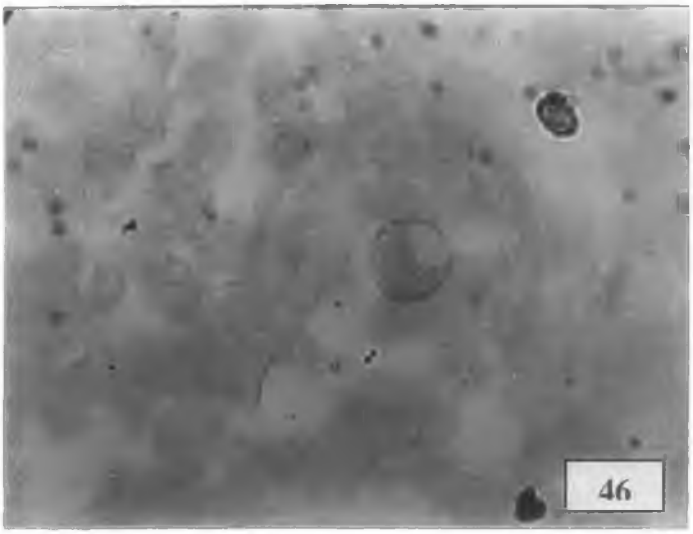
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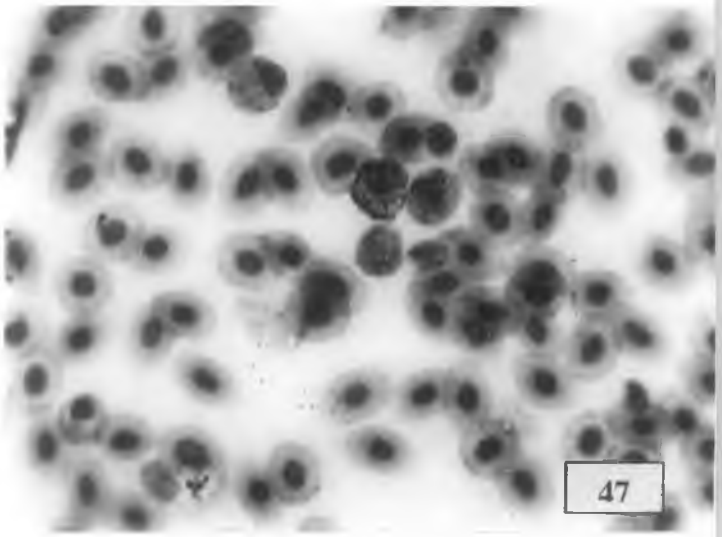
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Neutrophil

The nuclei of neutrophils were oval in shape and rarely did they appear bilobed in structure. The nuclei took light magenta colour while the cytoplasm appeared light blue due to presence of some granular structures in the cytoplasm when stained with Lishman stain (Fig. 47). Presence of lipid droplets were detected in the cytoplasm of neutrophils when the slides were stained with Sudan Black B (Fig. 44).The neutrophils showed strong PAS reactivity. The cytoplasms of the cells showed the strongest reaction while the nuclei showed a very mild reaction (Fig. 48).

The average cell diameter of neutrophils was 15.53±2.27 µm while the average nuclei diameter was 9.6±1.97 µm resulting the n-c ratio = 1: 1.62

Basophils

Basophils were scanty in number. The size of the cells was almost similar to neutrophils but the nuclei were either centrally located or peripherally located. The cytoplasm showed a lot of granules and took bluish colour (Fig. 42). These cells were found slightly PAS positive.

The average cell diameter of the cells was 10.2±0.13 µm and the average nuclei diameter was 6.62±0.12 µm resulting the n-c ratio = 1: 1.54.

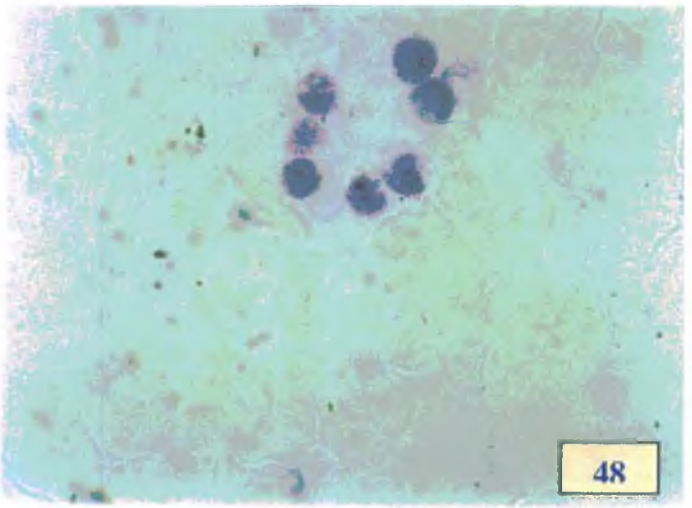
Eosinophils

Eosinophils were very rare in peripheral blood. The shape of the nucleus was irregular and the cytoplasm was full of acidophilic granules (Fig. 45). These cells were found PAS negative.

The average cell diameter of the cells was 17.3±2.31 µm and the average nuclei diameter was10.21 ±1.2 µm resulting the n-c ratio = 1: 1.69.

Fig.48: Blood smear of *C. mrigala* stained with PAS reaction shows the presence of neutrophils showing strong PAS reactivity.

Fig.49: Blood smear of *C. mrigala* stained with Leishman stain shows presence of a Plasma cell.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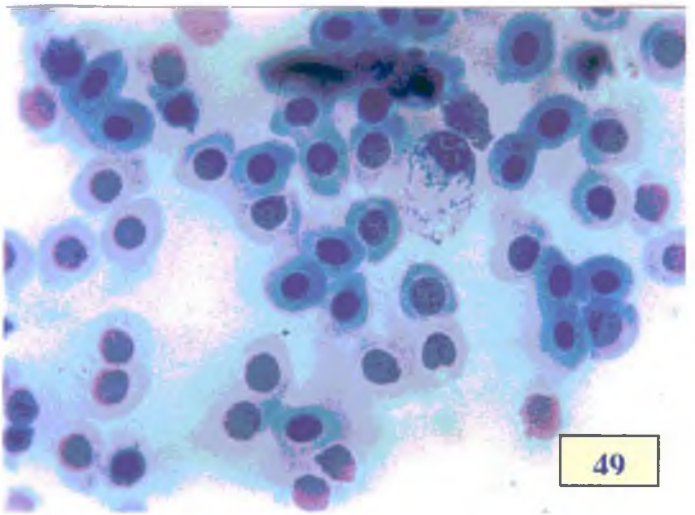
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Thrombocytes

Thrombocytes were seldom found in the peripheral blood of healthy *C. mrigala.* The nucleus was spindle shaped and deep magenta in colour surrounded by very thin cytoplasm which took no stain.

Plasma cells

A very few large cells were found in blood smears of healthy *C. mrigala.* These cells had an eccentric nucleus with a very low n-c ratio. The shape of the cells was oval-elliptical. The cytoplasm did take a very faint stain when stained with Romnowsky's stains. But the cytoplasm showed a fine reticulate structure (Fig. 49).

Table4. Average diameter of nucleus and cell along with nucleus cytoplasm ratio of different blood cells of healthy *C. mrigala.*

	N	Cell	n/c
Erythrocyte	2.64±0.3375	6.0±0.59	1: 2.27
Monocyte	10.33±1.84	17.23±2.46	1: 1.67
Neutrophil	9.6 ±1.97	15.53±2.27	1: 1.62
Lymphocyte			
1. Small	2.5±0.65	3.72±0.39	1: 1.48
2. Large	9.4±0.12	11.2± 0.42	1: 1.19
Basophil	6.62±0.12	10.2±0.13	1: 1.54
Eosinophil	10.21±1.2	17.3±2.31	1: 1.69

Mean ± S.D; n = 50

4.8. Cytochemical studies on erythropoiesis in healthy C. mrigala

The different stages of red blood corpuscle development as well as their morphological features are described below (Table 5).

Small lymphoid haemoblast or slh

The cells were small in size and completely round with a deeply stained nucleus. The nucleus took deep magenta colour in Graham-Knoll Benzidine (counter stained with Giemsa) (Fig. 50). The nucleus was surrounding by a thin rim of cytoplasm. The average diameter of the cells was 3.65±0.15μm and the n-c ratio was 1:1.2.

Basophilic erythroblast or be

These cells represented the next stage of development of erythrocytes. These cells were slightly larger size in comparison to small lymphoid haemoblasts. The average diameter of the cells was 4˙23±0.18μm and the average diameter of the nucleus was 3˙21±0.12μm. The n-c ratio of the cells was 1:1˙31. The nucleus was found less deeply stained compared to small lymphoid haemoblasts or slh.

Polychromatophilic erythroblasts or pe

These cells were characterized by the appearance of acidophilic areas within basophilic cytoplasm. These cells were larger than basophilic erythroblasts (Fig. 51). The average cell diameter was 4˙85±1.5μm and the average diameter of nucleus was 3˙0±0.12μm. The n-c ratio was 1:1˙6.

Acidophilic erythroblasts or ae

These cells represented the fourth stage of development of erythrocytes. The cytoplasm was acidophilic. The size of the cells was larger in comparison to that of other stages. The average diameter of the cells and nuclei were 5.98±1.13μm and 4.18±0.14μm respectively. The n-c ratio was 1:1˙43.

Young reticulocytes

These cells were characterized by the presence of homogenous mass of haemoglobin. The nucleus was oval in shape (Fig. 51). The average nucleus diameter was 2.63±0.13µm and the cell diameter was 7.2±1.2µm resulting the n-c ratio 1: 2.75.

Mature erythrocytes or mr

Mature erythrocytes were larger in size compared to that of young reticulocytes with an oval nucleus (Fig. 51). The average cell diameter was 6.0±0.13 while nucleus diameter was 2.64±0.01

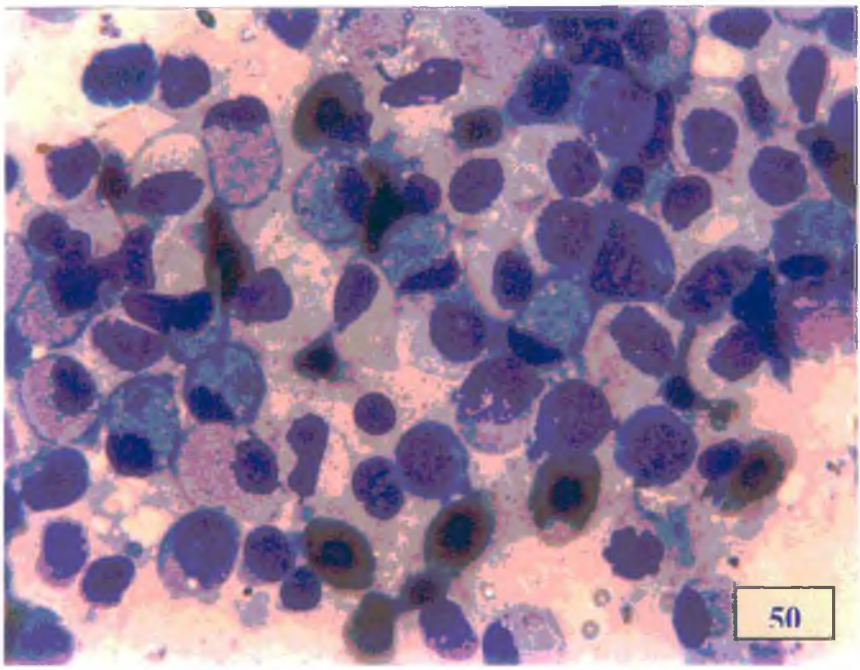
Table5. Measurement of nucleus and cell diameter and n-c ratio of six developmental stages of erythrocytes in healthy *C. mrigala.*

	Nucleus(µm)	**Cell diameter(µm)**	**n-c ratio**
Small lymphoid haemoblast or slh	3.04±0.10	3.65±0.15	1:1.2
Basophilic erythroblast or be	3.21±0.12	4.23±0.18	1:1.31
Polychromatophilic erythroblast or pe	3.0±0.12	4.85±1.5	1:1.6
Acidophilic erythroblast or ae	4.18±0.14	5.98±1.13	1:1.43
Young reticulocytes	2.63±0.13	7.2±1.2	1:2.75
Mature erythrocytes	2.64±0.01	6.0±0.13	1:2.27

Mean±S.D; n= 50

Fig.50: Tissue imprints of head kidney of healthy *C. mrigala* stained with Graham-Knoll Benzidine counter stained with Giemsa show different developmental stages of erythrocytes, like small lymphoid haemoblast or slh.

Fig.51: Tissue imprint of spleen of healthy *C. mrigala* shows the presence polychromatophilic erythroblast or pe, young reticulocytes and mature erythrocytes.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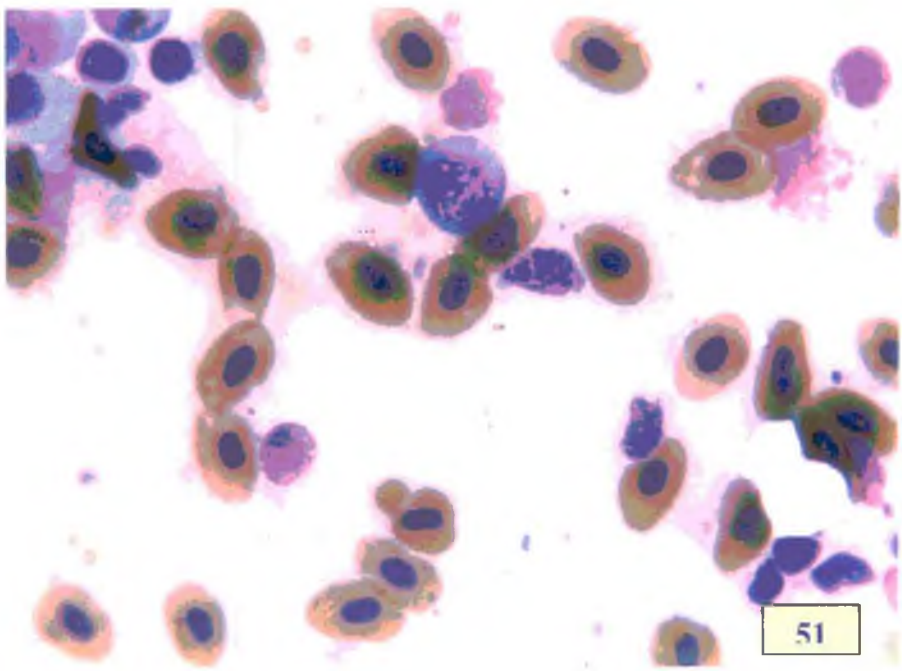
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4.9. Erythropoietic efficiency of haemopoietic organs of both healthy and EUS affected *Cirrhinus mrigala*

(Table 6) contains the data related with erythropoietic efficiency of head kidney and thymus in both healthy and EUS affected *C. mrigala.*

Healthy *C. mrigala* showed 3291±37.32 blast cells/mm^3 in the head kidney and 3275±66.14 blast cells/mm^3 in the thymus.

But EUS affected *C. mrigala* showed a value of 2695±42.72 blast cells/mm^3 in the head kidney and 2116.67±104.08 blast cels/mm^3 in the thymus.

The results of erythropoietic efficiencies of head kidney and thymus of both healthy and EUS affected *C. mrigala* clearly showed that erythropoietic efficiencies of head kidney and thymus of healthy fish were significantly ($p < 0.001$) higher in comparison to erythropoietic efficiencies of those organs in EUS affected fish.

Figs. 52 and 53 show the comparison among erythropoietic efficiencies of head kidney and thymus of healthy and EUS affected *C. mrigala.*

Table6. Erythropoietic efficiency of head kidney and thymus of healthy and EUS affected *C. mrigala.*

Fish *(Cirrhinus mrigala)*	**Wt. (gm)**	**Wt. of head kidney (gm)**	**No. of blast cells produced (cells/mm^3)**	**Wt. of thymus (gm)**	**No. of blast cells produced (cells/mm^3)**
Healthy	40±2.0	0.0203±0.0005	3291±37.32	0.0079±5.77	3275±66.14
EUS affected	42.167±2.021	0.214±0.001	2695±42.72*	0.0106±0.0012	2116.67±104.08*

Mean±S.D; n==3; * Significant at 0.001% level

Erythropoietic efficiency of head kidney and thymus of healthy and EUS affected *C. mrigala*

Fig. 52

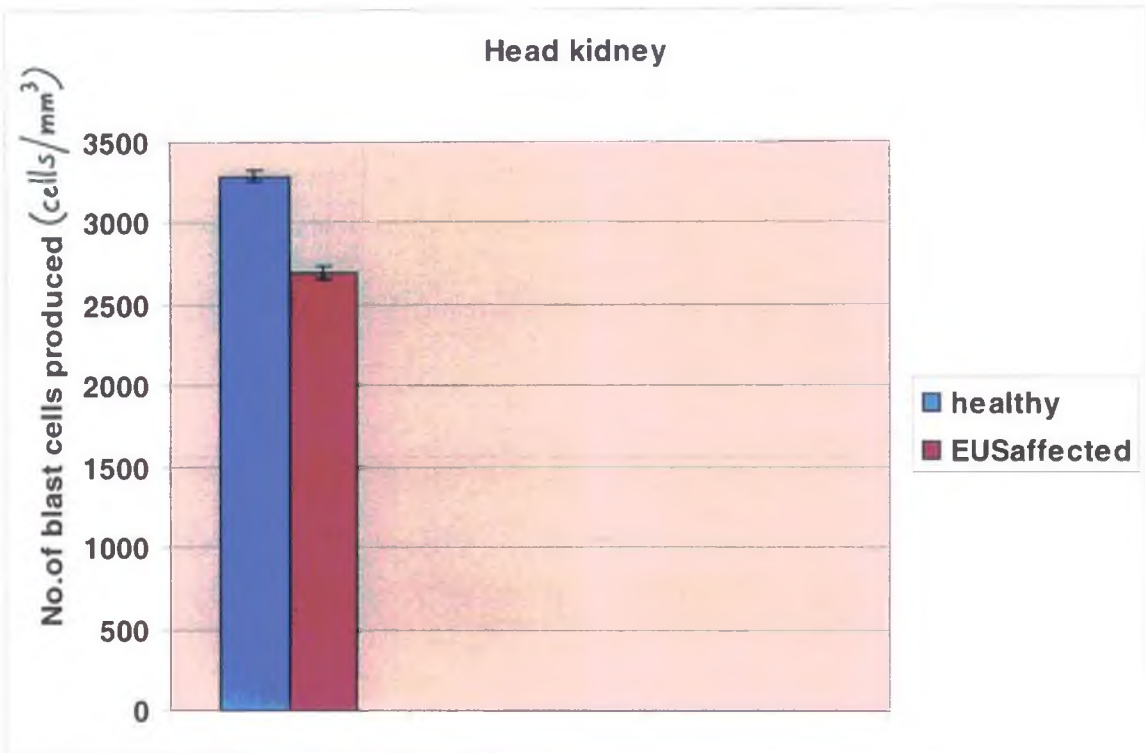
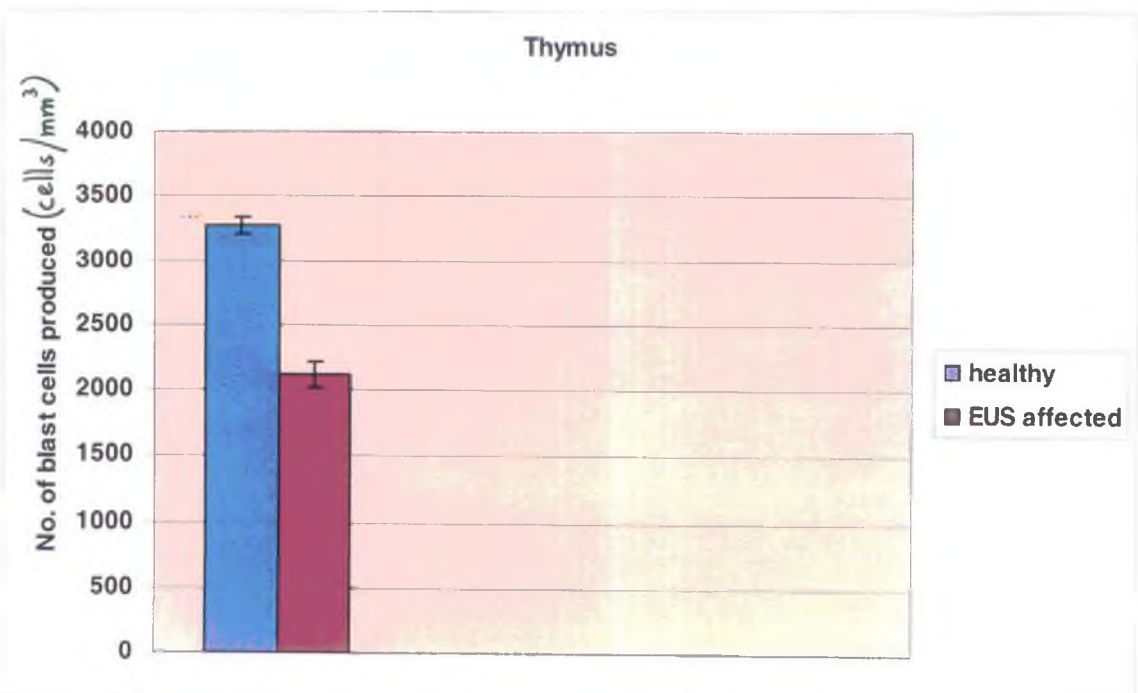


Fig. 53



4.10. Changes in the morphology of erythrocytes of naturally infected fishes

The result of the count of elliptical and oval shaped erythrocytes in the peripheral blood of healthy and EUS affected fishes are shown in Table 7. In peripheral blood of healthy *C. mrigala* the percent of elliptical erythrocytes was 79.6±3.647 while percent of oval erythrocytes was 20.4±3.647.

But in peripheral blood of EUS affected *C. mrigala* the percent of elliptical (Fig. 54) and while oval erythrocytes were 62.2±5.404 and 37.8±5.404 respectively.

The percent of oval (Fig. 55) shaped erythrocytes in peripheral blood of EUS affected *C. mrigala* was significantly higher ($P<0.001$) compared to that in peripheral blood of healthy *C. mrigala.* Fig. 56 shows the comparison between the percentage of elliptical and oval shaped erythrocytes in peripheral blood of healthy and EUS affected *C. mrigala.*

Table7. Count of Elliptical and Oval erythrocytes in peripheral blood of healthy and EUS affected fish

Species ***(Cirrhinus mrigala)***	**Percent of Elliptical**	**Percent of Oval**
Healthy	79.6±3.647	20.4±3.647
EUS affected	62.2±5.404	37.8±5.404*

Mean ±S.D; n=3; *Significant at 0.001% level

Count of elliptical and oval shaped erythrocytes in peripheral blood of healthy and EUS affected *C. mrigala*

Fig. 56

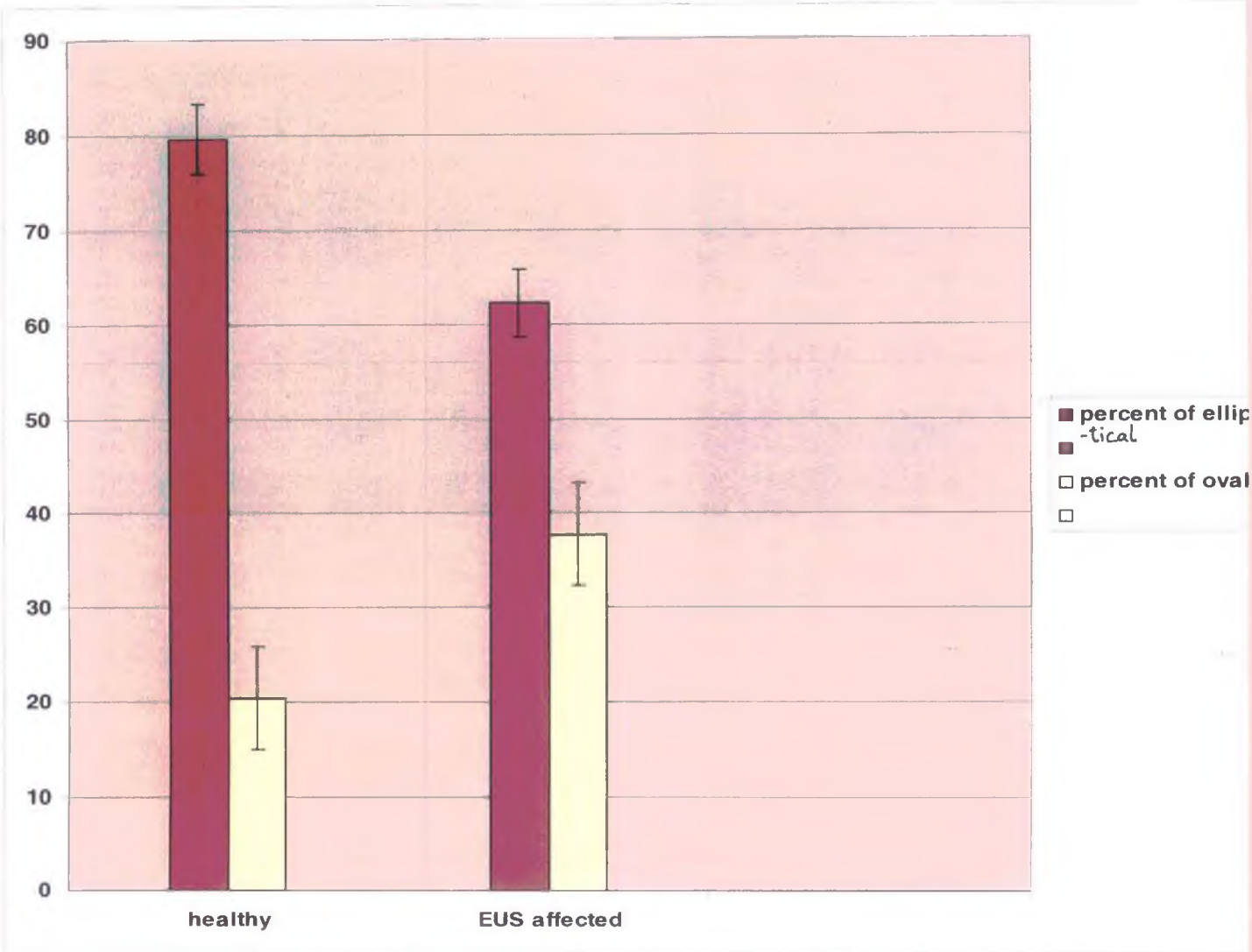
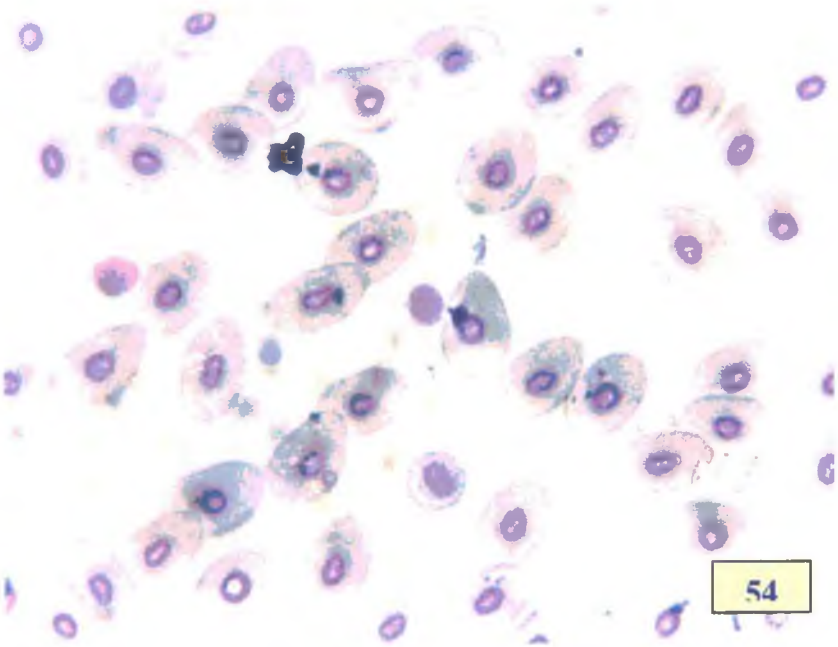


Fig.54: Blood smear of healthy *C. mrigala* stained with Leishman stain shows that almost all the erythrocytes are of mature stage i;e elliptical in shape.

Fig.55: Blood smear of EUS affected *C. mrigala* stained with Leishman stain show the presence of huge number of immature erythrocytes with oval shape.



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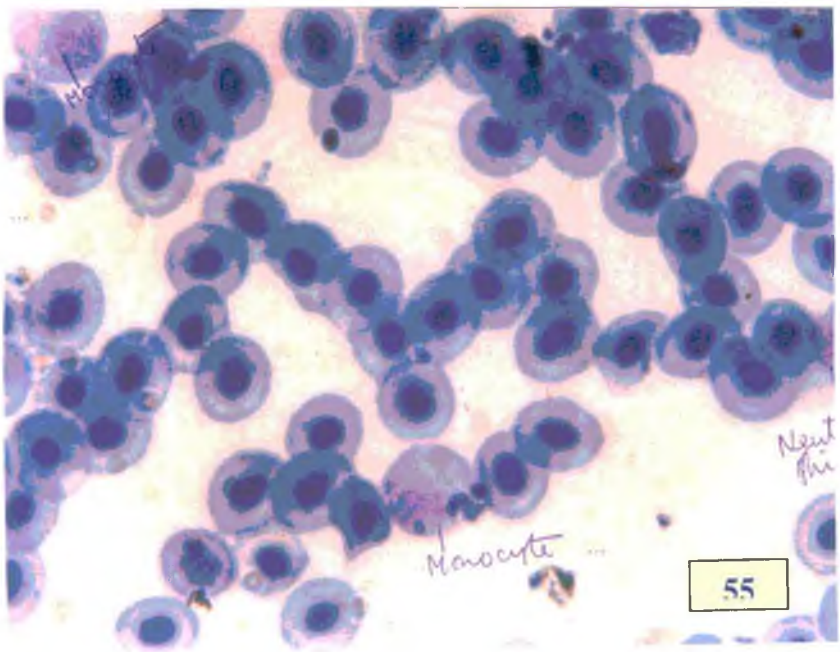

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4.11. Total erythrocyte count in peripheral blood of healthy and EUS affected *Cirrhinus mrigala*

The result of the total erythrocyte count (TEC) in healthy and EUS affected fishes are shown in Table 8.

In peripheral blood of healthy *C. mrigala* the number of total erythrocyte was $4.5\pm0.32\times10^6/mm^3$ while in peripheral blood of EUS affected *C. mrigala* it was only $1.44\pm0.16\times10^6/mm^3$

The TEC in EUS affected fishes was significantly ($p<0.001$) lower in comparison to that of healthy fishes. Fig. 57 shows the comparison of the total erythrocyte count in peripheral blood of healthy and EUS affected *C. mrigala.*

Table8. Total erythrocyte count ($\times10^6/mm^3$) in peripheral blood both healthy and EUS affected *C. mrigala*

Healthy fish	4.5±0.32
EUS affected fish	1.44±0.16*

Mean±S.D; n=10; * Significant at 0.001%

4.12. Total leucocyte count of healthy and EUS affected fishes

Total leucocyte count of healthy and EUS affected fishes are shown in Table 9.

Total leucocyte count in peripheral blood of healthy *C. mrigala* was 26.93±10.13 while total leucocyte count in peripheral blood of EUS affected *C. mrigala* was 90.93±13.86. Fig. 58 shows the comparison of total leucocyte count between healthy and EUS affected *C. mrigala.*

Table9. Total leucocyte count ($\times 10^3/mm^3$) in peripheral blood of both healthy and EUS affected *C. mrigala*

Healthy fish	26.93±10.13
EUS affected fish	90.93±13.86*

Mean ± S.D; n = 10; * Significant at 0.001%

4.13. Haematological studies of healthy *C. mrigala* throughout the year (2007)

Table 10 showed the total amount of haemoglobin, total R.B.C. count or (TRC), total lymphocyte count or (TLC) and differential count of three subpopulations of leucocytes namely monocytes, neutrophils and lymphocytes in peripheral blood of healthy *C. mrigala* of an average weight 88.75±21.11 gm.

Haemoglobin content in peripheral blood of healthy *C. mrigala* varied from 5.16±0.15 to 6.23±0.25 gm/100mL blood throughout the year. From January to May (2007) the haemoglobin content in peripheral blood was between 6.11±0.10 and 6.2±0.10 (Fig. 59). From June to September it showed a trend of decrease and finally in September it went down to 5.16±0.29 gm/100mL level. From October onwards the haemoglobin content started showing an increase in amount and reached upto 6.23±0.25 gm/100mL during December.

Total R.B.C count in peripheral blood varied from 3.5±0.2 $\times 10^6/mm^3$ to 5.1±0.1 $\times 10^6/mm^3$ throughout the year. Like haemoglobin content from January to May (2007) TEC was 4.4±0.26 to 4.63±0.15 $\times 10^6/mm^3$ (Fig. 60). Similarly from June to September it showed a trend of decrease and went down to 3.5±0.2 gm/100mL during August. From

Total erythrocyte and leucocyte count in peripheral blood of both healthy and EUS affected *C. mrigala*

Fig. 57

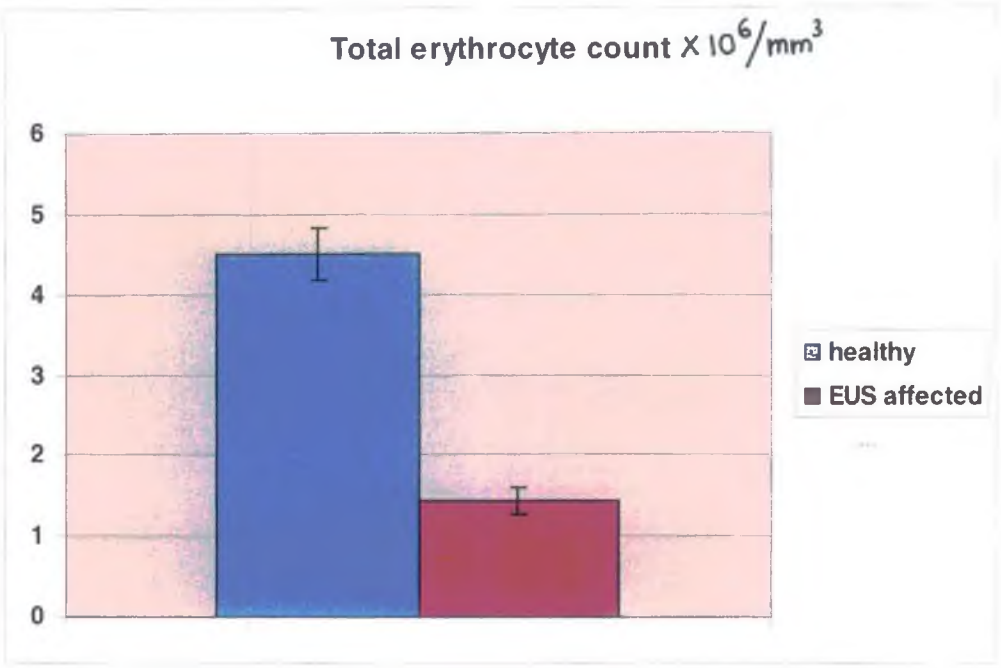


Fig. 58

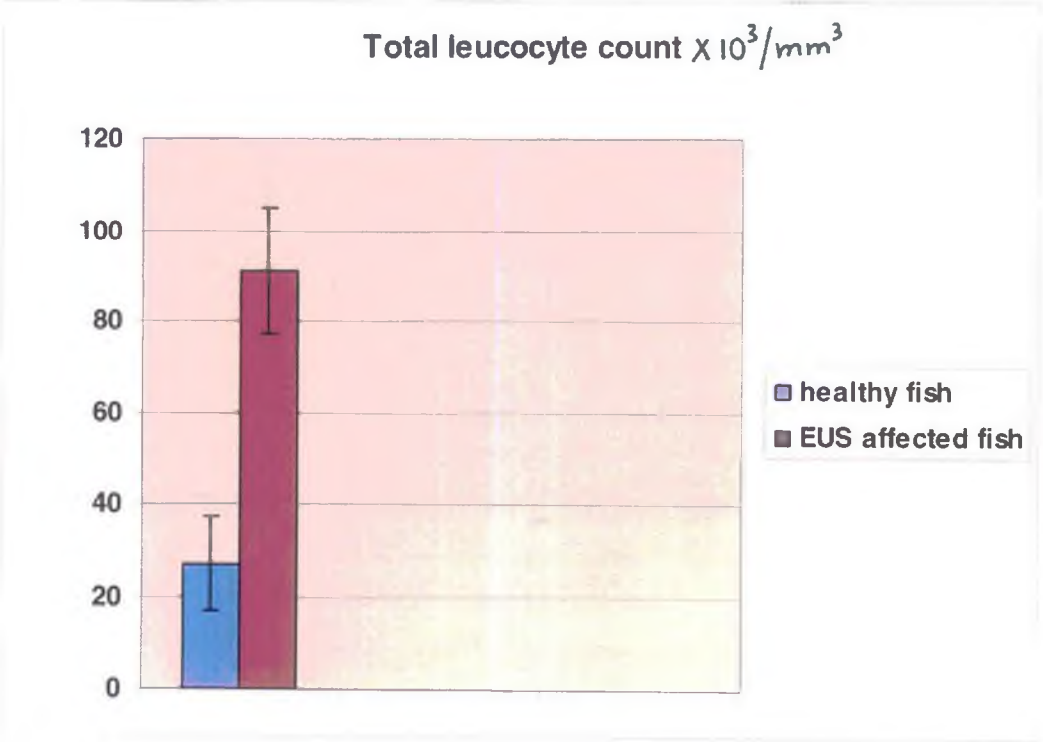


Table10. Hb content, total erythrocyte count (TEC), total leucocyte count (TLC) and differential count of lymphocytes, neutrophils, monocytes in peripheral blood

Month	Water temp. (°C)	Avg. wt (gm)	Hb content (gm/100mL)	TEC ($\times 10^6/mm^3$)	TLC ($\times 10^3/mm^3$)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)
Jan	16	60±5.00	6.11±0.10	4.63±0.15	22±4.58	59.66±1.53	35.33±1.53	5±1.0
Feb	19	65±5.00	6.2±0.10	4.4±0.26	24.66±3.93	55.3±2.57	35.33±3.05	9±4.0
Mar	24	70±8.89	6.2±0.20	4.6±0.36	23.93±3.58	51.66±2.52	35.33±3.51	13±1.15
April	27	80±20.0	6.1±0.10	4.4±0.2	24.46±4.21	47.66±1.53	36±1.0	16±3.5
May	30	70±12.12	6.16±0.15	4.2±0.2	23.9±4.53	41.33±1.53	38.66±1.53	20±1.15
June	30	80±15.00	5.96±0.15	4.0±0.2	22.4±3.84	44.6±1.64	36.03±1.53	19.33±1.15
July	31	90±20.00	5.5±0.30	3.8±0.2	23.06±3.72	45±1.53	30.33±2.52	25±2.65
Aug	30	100±8.66	5.16±0.15	3.5±0.2	23.5±4.24	60±3	20±1.0	20±1.0
Sept.	25	110±13.23	5.16±0.29	3.8±0.21	22.73±4.71	58±2.0	25±5.29	17±2.0
Oct	25	100±9.66	6.0±0.21	4.4±0.1	22.73±3.58	56.33±2.08	30±2.0	14±2.65
Nov	20	120±20.00	6.1±0.10	4.9±0.1	22.1±3.93	54.33±2.52	33.66±1.53	12±1.0
Dec	17	120±26.46	6.23±0.25	5.1±0.1	21.53±3.46	56±2.0	33±2.52	11±1.73

Data for each index are mean of three (n = 3)

Haematological studies of healthy *C. mrigala* throughout the year (2007)

Fig. 59

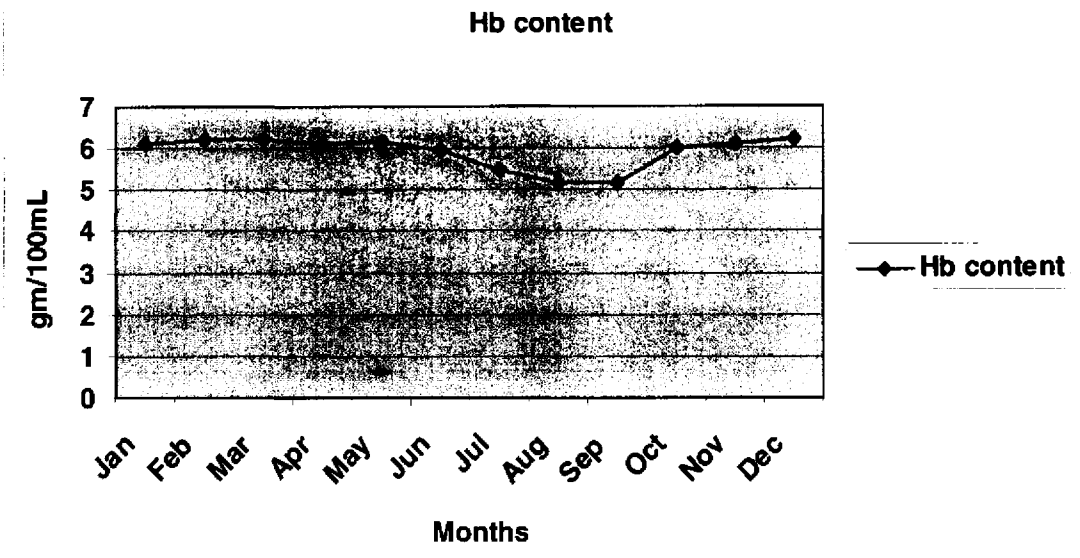


Fig. 60

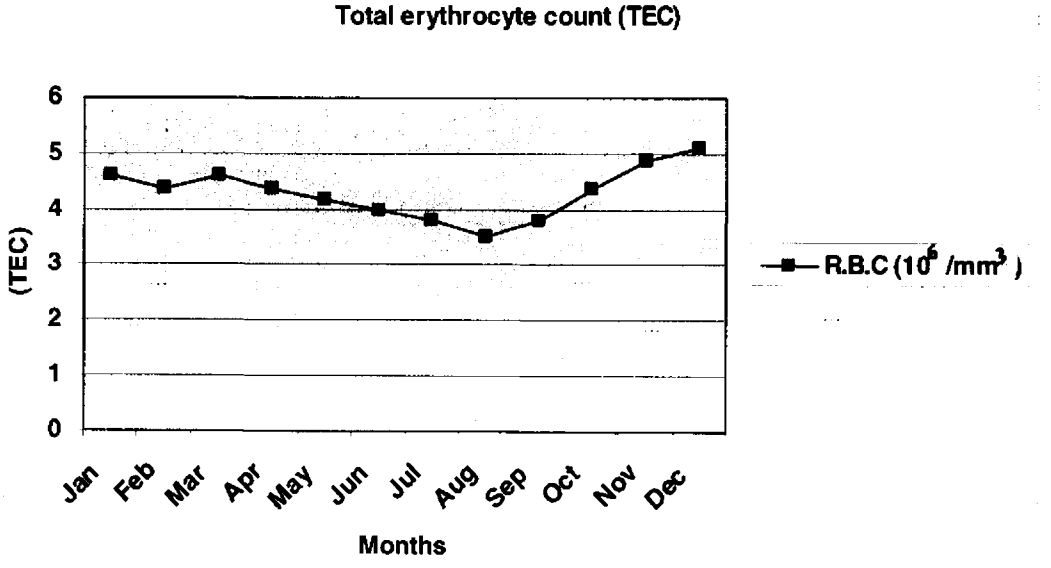


Fig. 61

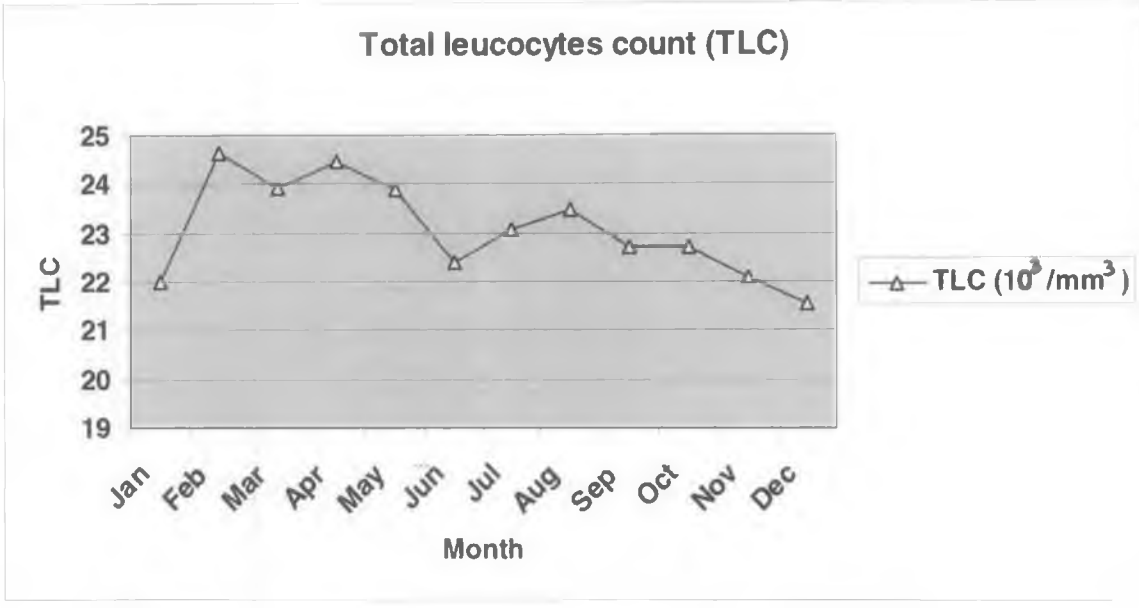

Total leucocytes count (TLC)
TLC
25
24
23
22
21
20
19
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
TLC (10³ /mm³)

Fig. 62

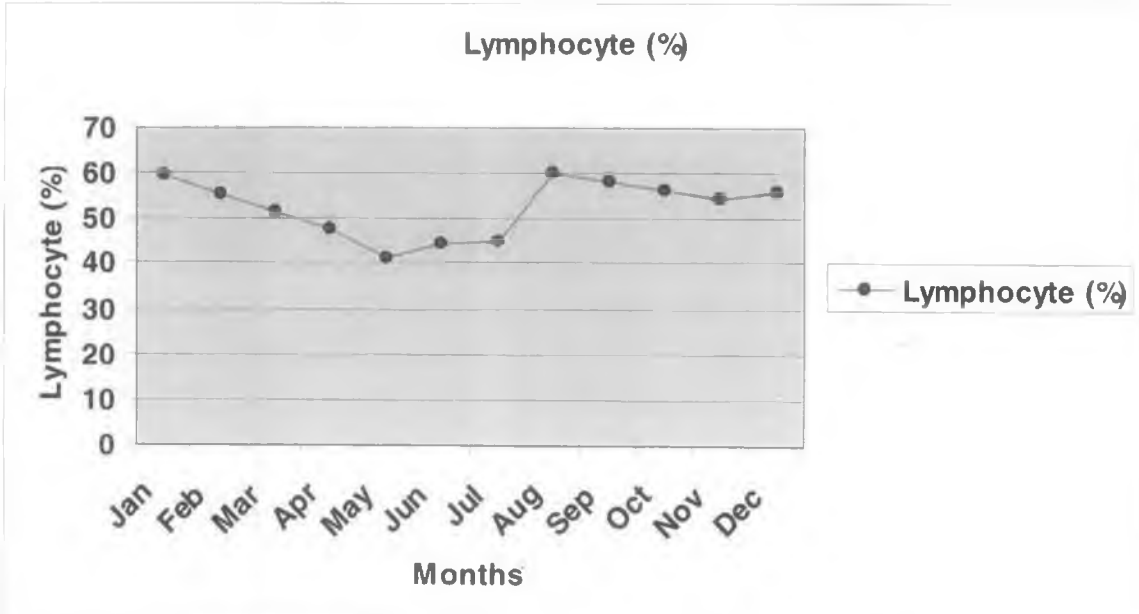

Lymphocyte (%)
Lymphocyte (%)
70
60
50
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months
Lymphocyte (%)

Fig. 63

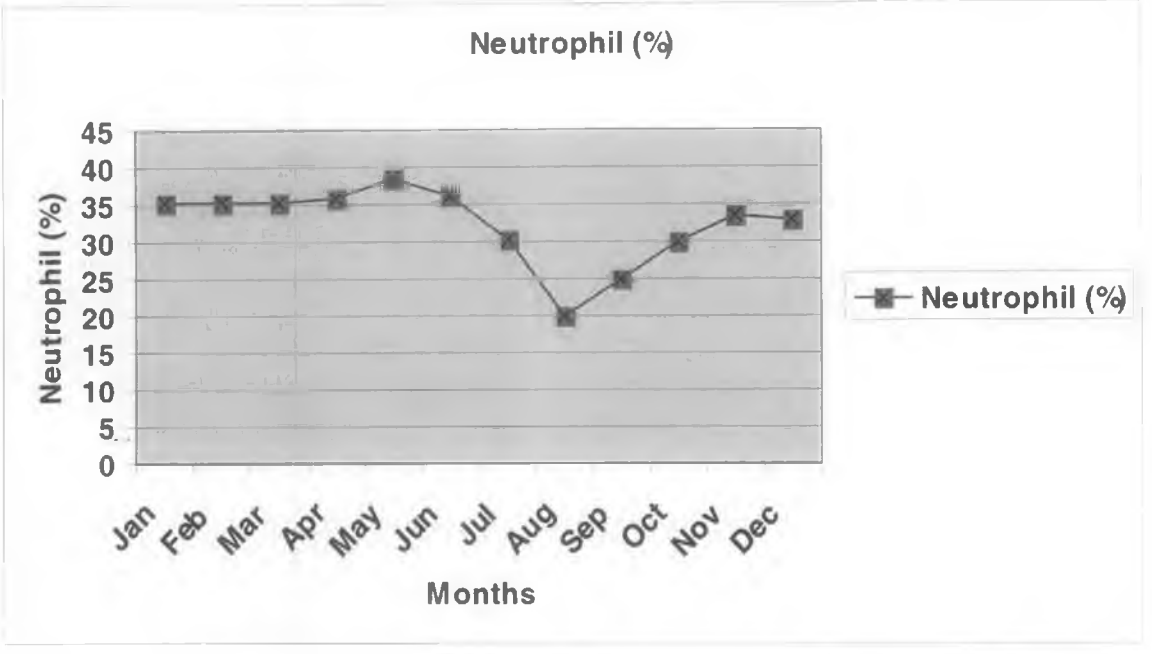
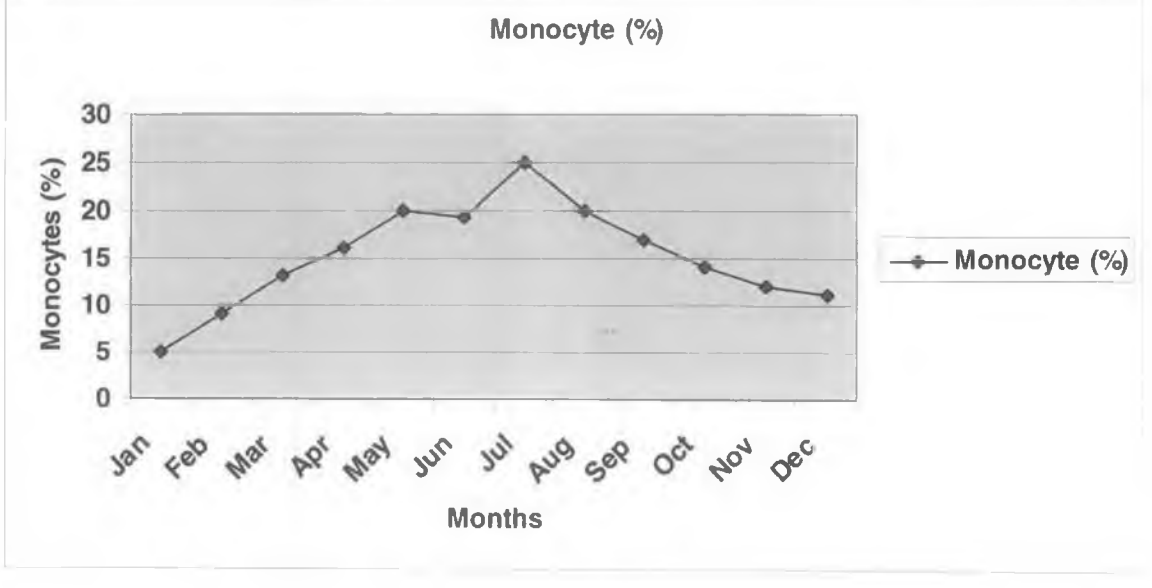

Neutrophil (%)
Neutrophil (%)
45
40
35
30
25
20
15
10
5
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months
Neutrophil (%)

Fig. 64


Monocyte (%)
Monocytes (%)
30
25
20
15
10
5
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months
Monocyte (%)

October it started showing a tendency to increase. Total leucocyte count in peripheral blood of healthy *C. mrigala* varied between $21.53\pm3.46\times10^3/mm^3$ and $24.66\pm3.93\times10^3/mm^3$ year round (Fig. 61).

Differential count of lymphocytes varied from 41.33±1.53% to 60±3% (Fig. 62). From January to June the percentage started to decline. From July onwards the percentage started increasing and varied between 45±1.53% to 60±3%.

Differential count of neutrophils varied between 20±1.0% and 38.66±1.33% (Fig. 63). From January to March, the differential count was almost unaltered. From April to June the differential count increased marginally. From July to August the differential count decreased being the lowest in August in 20±1.0%. But during November to December it increased to 33±2%.

Differential count of monocytes varied from 5 to 25% in peripheral blood of healthy *C. mrigala*. From January to July it started showing a steady increase then from August to December it showed a steady decline in percentage (Fig. 64).

4.14. Differential count of leucocytes in tissue imprints prepared from three lympho-heamopoietic organs of EUS affected *Cirrhinus mrigala*

Deferential count of different subpopulations of leucocytes in tissue imprints of three lymphohaemopoietic organs of EUS affected *C. mrigala* are shown in Table11.

In the tissue imprints of head kidney, spleen and thymus of EUS affected *C. mrigala* the number of basophils were very rare. In head kidney it was 1% while in spleen and thymus it was 0.66% and 1.33% respectively (Fig. 65).

The monocytes were the highest in head kidney (11±4.58%) while in spleen and thymus the percentage was 8±5% and 7.5±3.5% respectively.

Lymphocytes were found highest in number (Fig. 66). In head kidney it was 54±1.0 percent and in spleen it was 51.35±3.51 percent while in thymus it was 59.29±8.62 percent (Fig. 67).

Neutrophil was 34.66±3.21%; in head kidney 39±4.21% in spleen and 30.24±6.08% in thymus (Fig. 68).

Eosinophils were 1.66±1.0%; 2.33±0.5% and 1.33±0.33% in head kidney, spleen and thymus respectively (Fig. 69).

There was no significant difference in the differential count of subpopulations of leucocytes of three lymphohaemopoietic organs of EUS affected *C. mrigala.*

Table11. The differential count of different subpopulations of leucocytes in three lympho-heamopoietic organs of EUS affected *C. mrigala*

Different leucocytes	**Basophil**	**Monocyte**	**Lymphocyte**	**Neutrophil**	**Eosinophil**
Head kidney	01	11±4.58	54±1.0	34.66±3.21	1.66±1.0
Spleen	0.66	8±5	51.35±3.511	39±4.51	2.33±0.5
Thymus	1.33	7.5±3.51	59.29±8.62	30.24±6.08	1.33±0.33

Mean±S.D; n= 3.

4.15. Differential count of different subpopulations of leucocyte in peripheral blood of EUS affected *Cirrhinus mrigala*

The differential counts of subpopulation of leucocytes in the peripheral blood of EUS affected *C.* mrigala are shown in Table 12.

The differential count of different subpopulations of leucocytes in three lympho-haemopoietic organs of EUS affected *C. mrigala*

Fig. 65

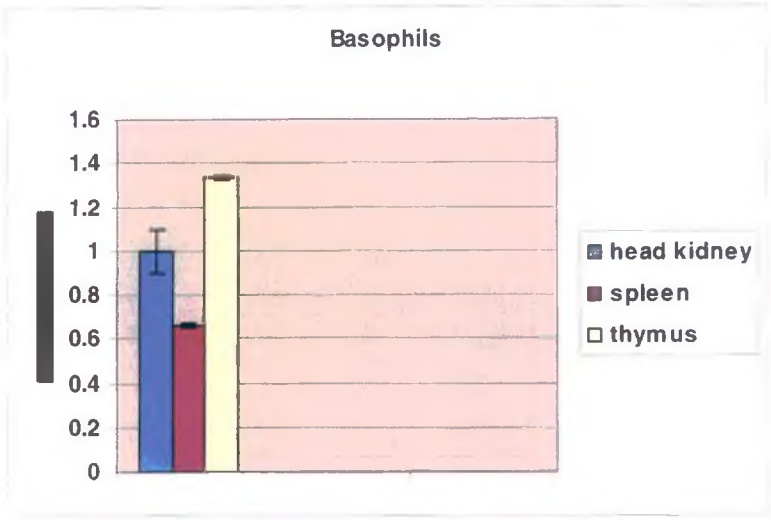
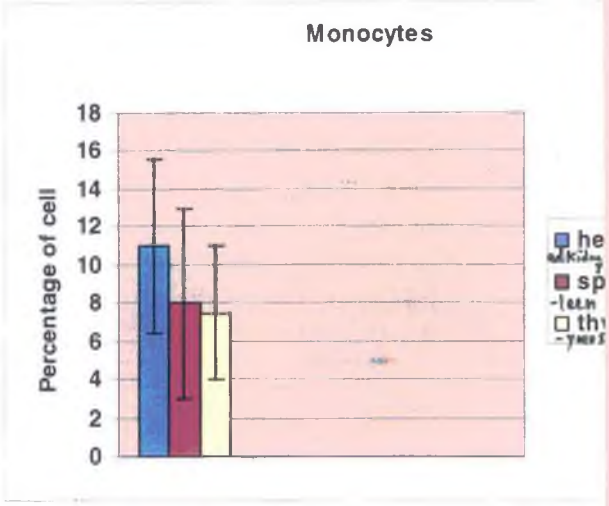


Fig. 66



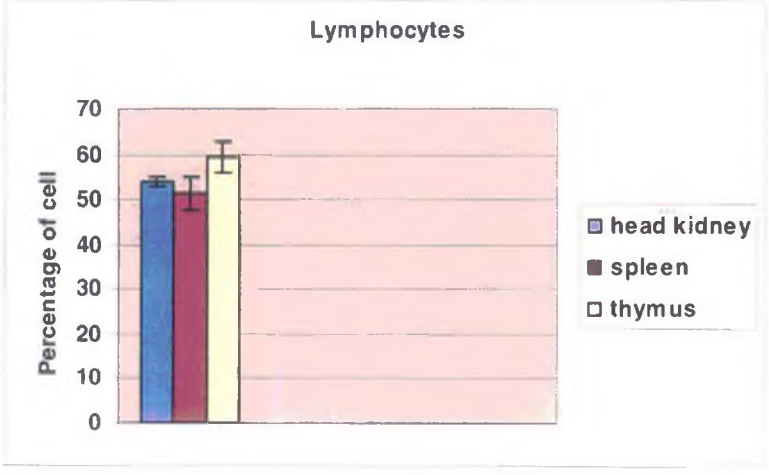


Fig. 67

Fig. 68

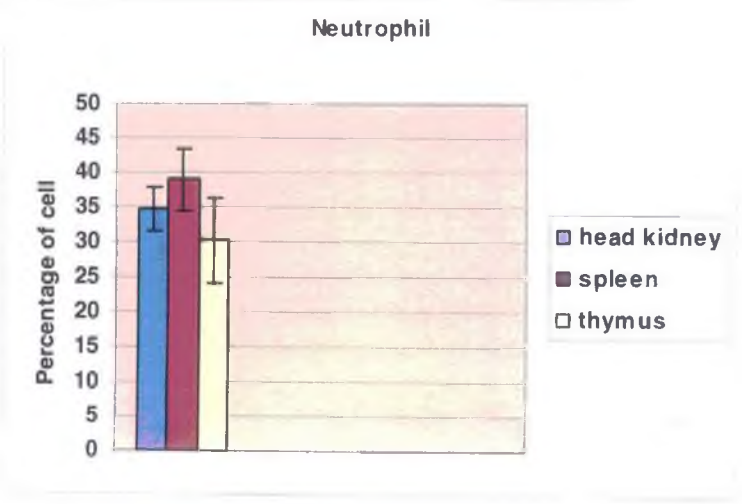
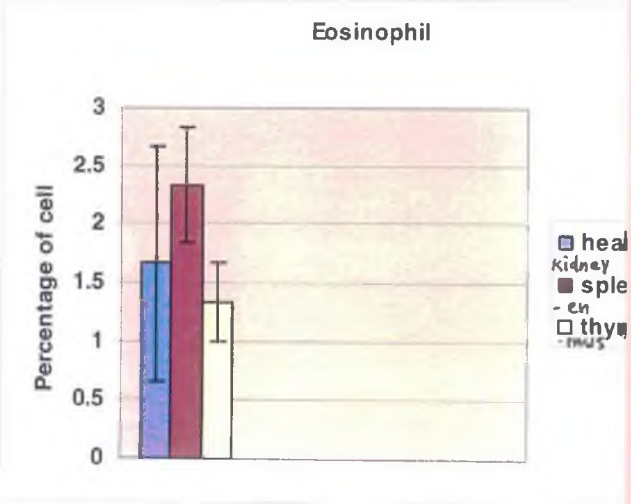


Fig. 69



Lymphocytes were present in peripheral blood of EUS affected *C. mrigala* at the highest percentage i; e 65.0±9.47 but neutrophils were present in only 20.43±6.37 percent. Monocytes were present in 12.17±5.33 percent and basophils and eosinophils both were present in 1±0% (Fig. 70).

Table12. Differential count of different subpopulations of leucocyte in peripheral blood of EUS affected *C. mrigala*

Eosinophil	Monocyte	Lymphocyte	Neutrophil	Basophil
1±0	12.17±5.33	65.0±9.47	20.43±6.37	1±0

Mean±S.D; n=10

4.16. Studies on immunological status of healthy *C. mrigala* year round

Table13 shows the number of plaques/10^6 W.B.C produced from cell suspension of two lymphohaemopoietic organs, head kidney and thymus during three seasons.

During summer number of plaques produced in head kidney was 689±8.54 while in thymus it was 482.33±10.78.

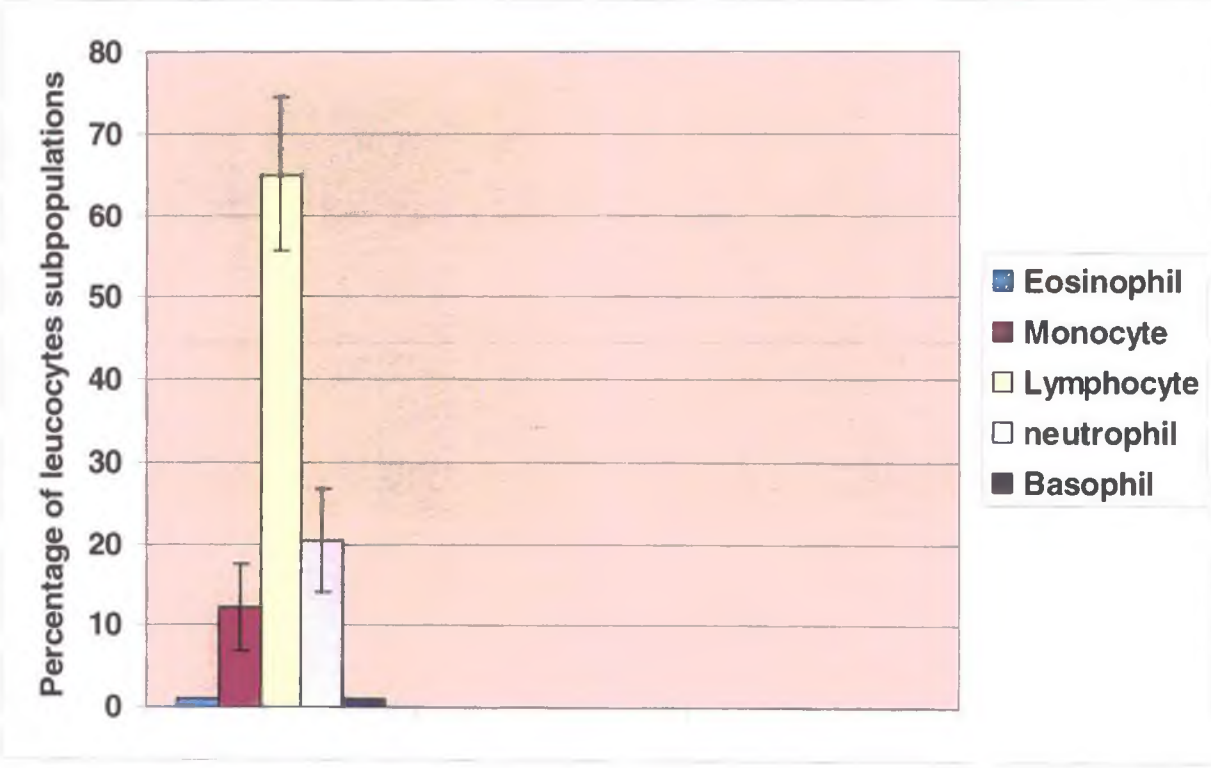
During rainy season, the number of plaques produced in head kidney was 905.88±8.87 while in thymus it was 592.88±43.86.

During winter, the number of plaques produced in head kidney was 530.33±19.19 while in thymus was 410.33±20.75 (Fig. 71).

In case of plaque forming cells assay, it was also observed that during winter months i; e from December to February when water temperature dropped significantly

Differential count of different subpopulations of leucocyte in peripheral blood of EUS affected *C. mrigala*

Fig. 70



the number of antibody secreting cells also dropped drastically. But during rainy season, though water temperature dropped slightly compared to the summer but number of antibody secreting cells increased.

The agglutinating antibodies produced after injection of antigen, SRBC or sheep red blood cells were found 2- mercaptoethanol sensitive, indicating that they were equivalent to the IgM of mammalian species.

Table13. The number of plaques/10^6 W.B.C produced from cell suspension of two lymphohaemopoietic organs, head kidney and thymus in three seasons

Season	Water temperature (°C)	Head kidney	Thymus
Summer	24°C-28°C	689±8.54	482.33±10.78
Rainy	22°C-28°C	905.88±8.87	592.88±43.86
Winter	16°C-22°C	530.33±19.19	410.33±20.75

* Data for each index are mean of three fishes. WBC= White Blood Cells. n=3

4.17. Measurement of amount of antibody produced throughout the year (2007) against (0.2 ml) Sheep Red blood cells or SRBC

Table 14 shows the amount of antibody produced against a fixed amount of antigen, Sheep Red Blood Cells measured by haemagglutination titre test (HA) on 5^{th} day and 10^{th} day after injection throughout the year (Figs. 72, 73, 74 and 75).

The number of plaques/10^6 W.B.C produced from cell suspension of two lympho-haemopoietic organs, head kidney and thymus in three seasons

Fig. 71

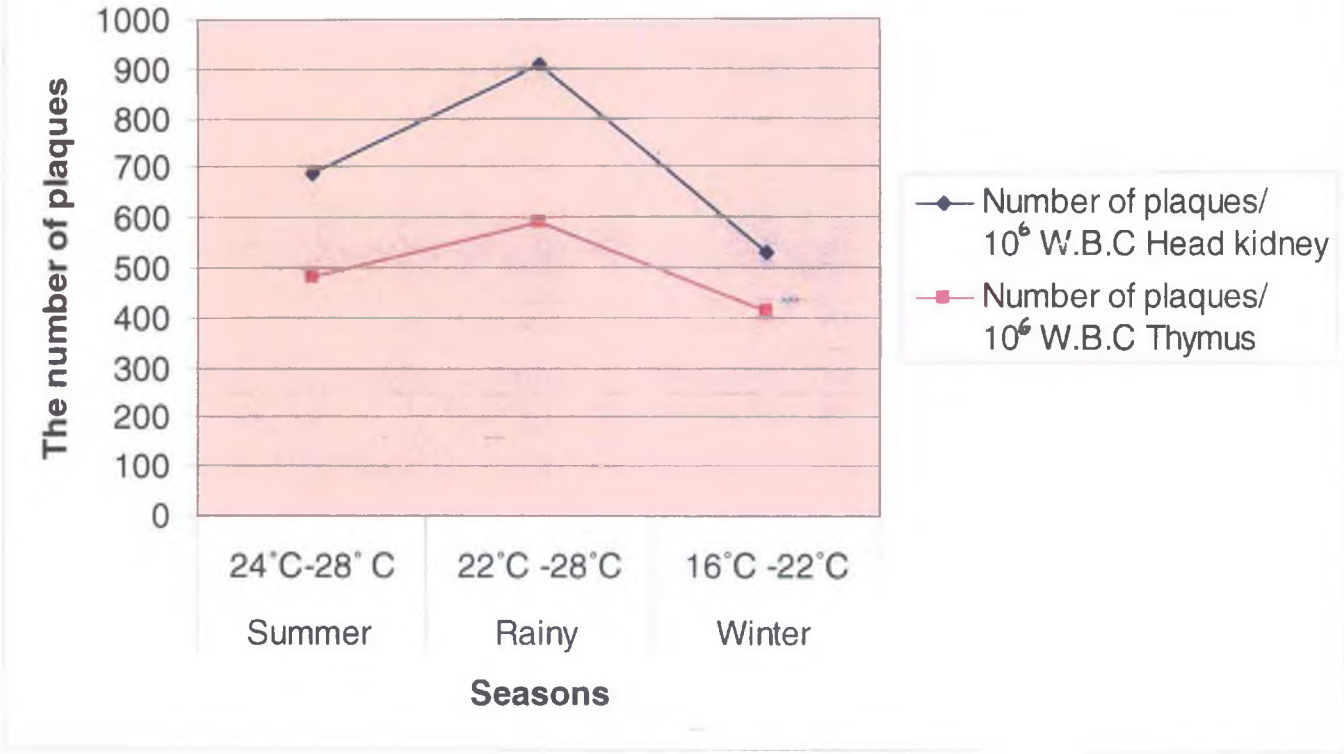
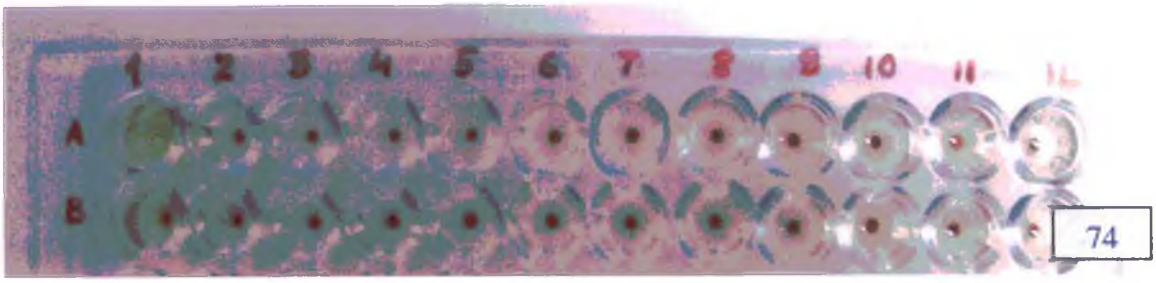
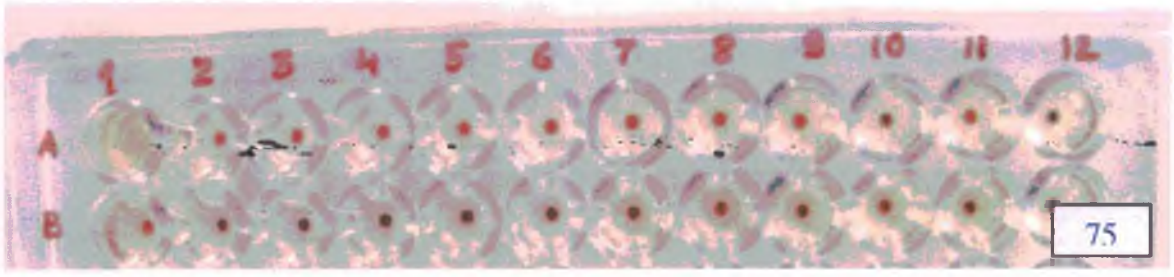


Fig.74: Series 'A' showing reaction in 1st well but series 'B' showing no reaction as 2-Merceptoethanol was added.

Fig.75: Series 'A' showing reaction in 1st well but series 'B' showing no reaction as 2-Merceptoethanol was added.

Fig.76: Series 'A' showing reaction upto 4th well while series B showing reaction upto 5th well.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
74


1 2 3 4 5 6 7 8 9 10 11 12
A
B
75


76

During January when the water temperature was the lowest (16-18°C) the amount of antibody production was also the lowest showing the reaction only in the 1st well in both occasions.

But in February, when the temperature increased (22-24°C) the amount of antibody production also increased showing reaction in 1st and 2nd wells on 5th day after injection and 1st, 2nd and 3rd wells on 10th day after injection .

During July and August the amount of antibody production was highest showing reaction upto 3rd well on 5th day after injection and upto 5th well on 10th day after injection and the water temperature was highest (28-30°C). Then with the decrease of water temperature the amount of antibody production also decreased being the lowest during December.

The results obtained from H.A tests showed clearly that as the temperature of water during winter season dropped the amount of antibody produced also dropped suggesting a partial suppression of immune response of fish.

The amount of antibody production by healthy *C. mrigala* against 0.2 mL of 25% SRBC throughout the year on 5th and 10th day after injection

Fig. 72

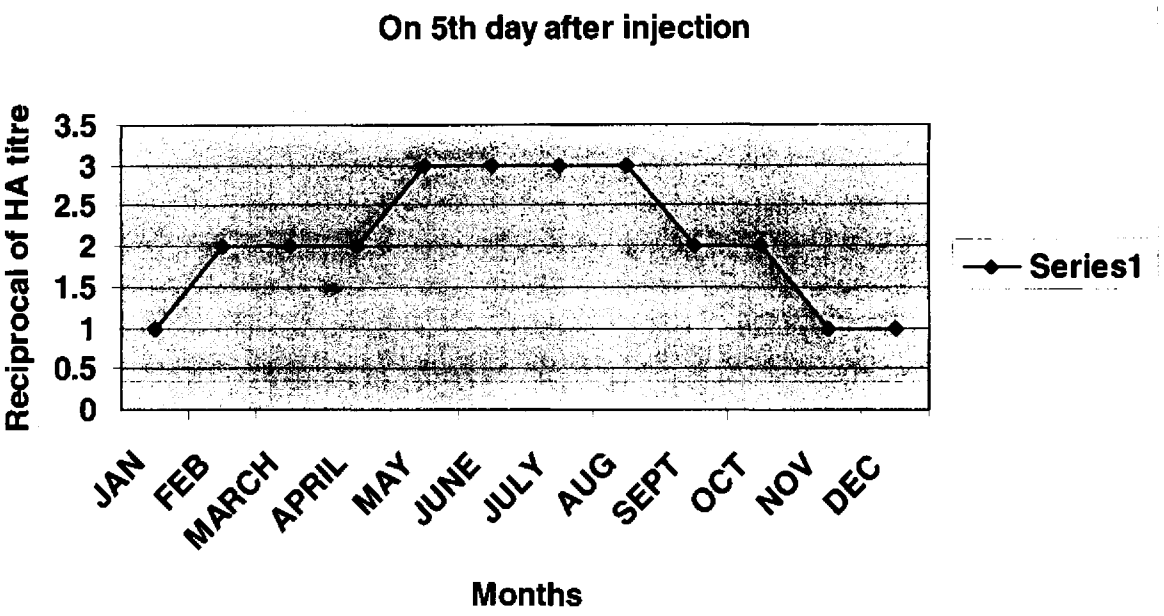


Fig. 73

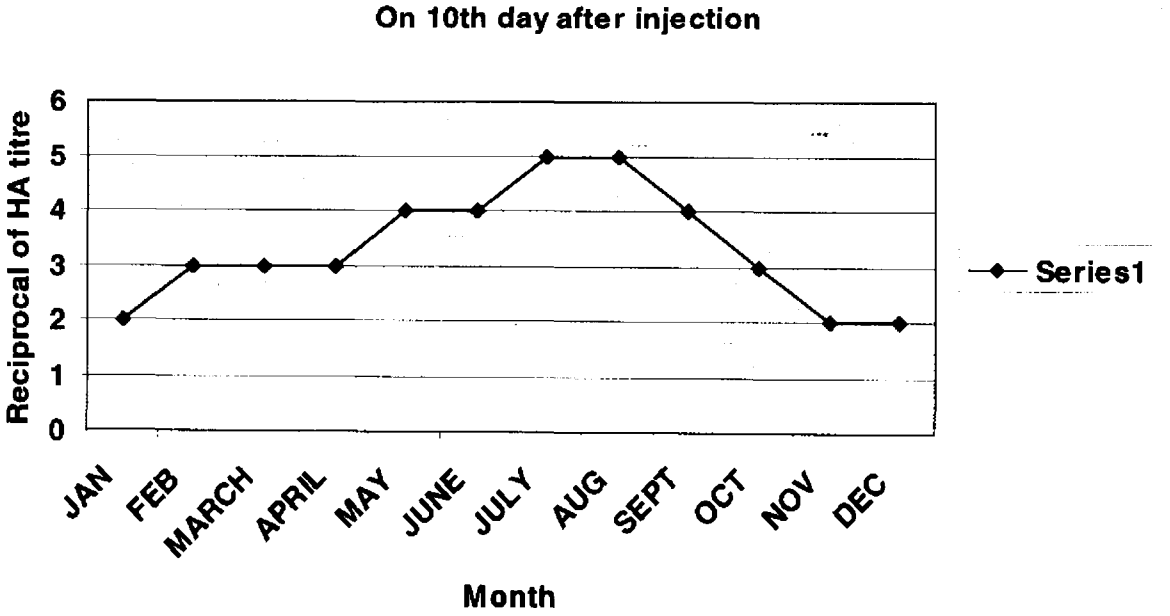


Table14. The amount of antibody production by healthy *C. mrigala* against 0.2 ml of 25% SRBC throughout the year (2007) on 5th day and 10th day after injection.

Month	Water Temp. (°c)	On 5th day after injection	10th day after injection
Jan	16-18	O	OO
Feb	20-22	OO	OOO
March	24-26	OO	OOO
April	24-26	OO	OOO
May	24-26	OOO	OOOO
June	26-28	OOO	OOOO
July	28-30	OOO	OOOOO
Aug	28-30	OOO	OOOOO
Sept	26-28	OO	OOOO
Oct	22-24	OO	OOO
Nov	20-21	O	OO
Dec	19-21	O	OO

Data for each index are mean of three fishes (n=3)

O = 1st well; OO = 1st and 2nd well; OOO = 1st to 3rd well; OOOO = 1st to 4th well; OOOOO = 1st to 5th well.