Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Microsc. Microanal. Res.

Haematological Profiles and Histological Investigations of Two Freshwater Fishes: Nile Tilapia (*Oreochromis niloticus*) and Hybrid Catfish (*Clarias macrocephalus* x *Clarias gariepinus*)

Duangkamolwan Kobkanta[1], Wararut Buncharoen[2], Supap Saenphet[2,*]

[1] The Royal Project Foundation, Muang Chiang Mai, Chiang Mai Province, 50200, Thailand
[2] Department of Biology, Faculty of Science, Chiang Mai University, Muang Chiang Mai, Chiang Mai Province, 50200, Thailand

*Corresponding author's e-mail address: *bigsu.cmu@gmail.com (S. Saenphet)*

ARTICLE INFO

ABSTRACT

Water pollution degrades aquatic ecosystems and also adversely affects freshwater teleosts. To assess the health status of Nile tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*), which are economically important fishes in Thailand, blood profiles and histological changes in the vital organs were examined. Haematological characteristics were studied including total red blood cell count, white blood cell count, haematocrit, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and differential white blood cells. The occurrences of micronuclei, immature erythrocyte and nuclear abnormality were also measured. Histological changes were observed in liver and kidney under a light microscope. The results showed that the Nile tilapia had higher values of red blood cell indices than those of the hybrid catfish, but not red blood cell count and mean corpuscular haemoglobin concentration. In additional, Nile tilapia had more micronuclei and abnormal nuclei than the hybrid catfish. In contrast, the number of leukocytes in the hybrid catfish was higher than in Nile tilapia. Pathological alterations such as vacuolization, necrosis, haemorrhage, blood congestions and Kupffer cell aggregations were recognised in hepatic tissues. These histological changes were more severe in the hybrid catfish than those in Nile tilapia. Shrunken glomerulus, detached tubular cells, blood congestions and leukocyte infiltrations were found in renal tissues of both fishes. From our results we suggest that the alterations in haematological indices as well as in liver and kidney tissues of these fishes were the result of physiological responses to stress conditions in their habitats.

INTRODUCTION

Water pollution caused by rapid growth of industrialization and agriculturalization is a universally serious problem. Contamination in water bodies has created adverse effects on aquatic fauna, especially different species of fishes. Freshwater fishes can easily contact with pollution in their habitats either via respiratory system or through ingestion of small aquatic organisms. Moreover, contaminants can be absorbed into blood vessels in the gills, because fish gills are in direct contact with the water medium [1]. Exposure to toxic compounds in a short period of time can cause toxic accumulations in fish bodies and consequently produce deleterious effects. Although different fish species can tolerate and adapt their biochemical and physiological mechanisms against the adverse conditions, chronic exposures can induce pathological changes and subsequently cause mortality. Thereby, toxicological research is being done to monitor physiological and pathological changes in a wide range of teleosts in polluted environments.

Unfavorable alterations in the circulatory system or in the other organ systems can reflect the qualities of the water and health status of fishes. Investigations of haematological parameters in a number of fish species under different environmental conditions such as pollutants, transportation, hypoxia, parasitic infection and acclimation have been performed either to understand physiological responses or to detect pathological changes. Furthermore, blood parameters can be used as an indicator for nutritional and overall health status of fish [2-4]. The levels of haemoglobin and haematocrit as well as erythrocyte sizes are significant parameters for studying the transport capacity, availability and removal rate of oxygen in fish tissues [5]. The harmful impacts of pesticide contamination in rivers have been clearly noted in the blood of a variety of fishes. A recent report indicated that a decrease in numbers of erythrocytes and an increase in numbers of leukocytes were found in Mahseer fish (*Tor putitora*) exposed to cypermethrin, a synthetic pyrethroid insecticide [6]. Similar to cypermethrin,

deltamethrin, a pyrethroidal pesticide showed haemotoxicity in freshwater fish (*Channa punctatus*) cultured in the contaminated river for 45 days. The decreases in haemoglobin content, total erythrocyte count and pack cell volume as well as an elevation of leukocyte count were also markedly noted [7]. These changes reflect the deleterious effects of pesticide contaminations on the immune system of fish. Besides the harmful effects of pesticides, the impacts of malnutrition, acclimation, heavy metals, seasonal change and parasitic infection on fish bloods have been documented [8-10]. In addition, there is evidence that pollutants or hazardous chemicals can also induce mutations in a variety of fish species by altering haematopoiesis. The micronuclei and nuclear abnormalities have been detected in teleosts such as barbel, rainbow trout, brown trout, European eel and European minnow exposed to natural polluted water and mutagenic agents [11-14]. Therefore, micronucleus test can be used to assess the risk to fishes from hazardous compounds.

Besides being responsible for detoxification and homeostasis, the liver and kidneys can accumulate any toxic compounds and exhibit degenerations [15, 16]. Thus, the hepato-renal index is widely accepted as an effective biological indicator for determining the effects of stress in contaminated areas on freshwater fishes. Alterations in enzyme activities as well as histopathological changes can be found in these organs after toxic exposure [17, 18]. An accumulation of copper in bufferfish (*Poronotus triacanthus*) has been documented in various organs. A highest level of copper was found in liver, followed by kidney, gills and muscular tissues. Swollen mitochondria, leukocyte infiltrations, pyknotic nuclei, vacuolization, elevations in size and numbers of lipid droplets were represented in their hepatic tissues. Furthermore, dilations of endoplasmic reticulum and cell necrosis were found in proximal tubules. These severe damages could found in bufferfish after seven days of exposure to copper [19]. Similarly, *Anabas testudineus* Bloch fishes inhabiting in unused lignite mine suffered from their pathological changes. The histopathological findings revealed deterioration and telangiectasis of the gill filaments. Haemorrhage, blood congestion and necrotic cells were found in their hepatic tissues. Additionally, epithelial hypertrophy and aneurysms of renal tubules as well as shrinkage of glomerulus were found in the renal tissues [20]. These alterations were a consequence of low pH values and the presences of residue metals in the mine. Additionally, hepato-renal damages in fishes produced by stress conditions such as heavy metals, pesticides and toxic chemicals have been previously documented [21-24].

Taken together, investigations of haematological profiles combined with histology of vital organs could indicate physiological and health status of fishes to identify unfavorable conditions that cause stress, especially in fisheries. This study, therefore, evaluated haematological profiles and histological changes in hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) which are economically important fishes in Thailand. The results obtained from our study provide a basic knowledge for application in monitoring quality of the environment.

METHODOLOGY

Fish samples

Ten adults of *Oreochromis niloticus* (20-25 cm length) and ten adults of *Clarias macrocephalus* x *Clarias gariepinus* (25-30 cm length) were randomly purchased from a local market from Muang Chiang Mai, Chiang Mai Province, Thailand. They were quickly shifted to a laboratory and kept in glass aquaria with constant aeration and non-chlorinated water prior to collecting the samples. Blood samplings were done within the first three hours of transportation.

Collections of blood and tissue samples

A blood sample was collected from each fish by caudal vein puncture technique [25]. The blood samples were transferred into tubes containing an anticoagulant agent, ethylene diamine tetraacetic acid (EDTA) for haematological and micronucleus analysis. After blood collection, all fishes were sacrificed and their liver and kidneys were quickly dissected. These two organs were fixed in Bouin's solution for at least 24 h for histological examinations.

Haematological analysis

The anticoagulated bloods were mixed with Dacie's solution and Gower's solution for determining total red blood cells (RBCs) and total white blood cells (WBCs) counts respectively. The blood mixtures were loaded into Neubauer haematocytometer chambers. After differentiations, RBCs and WBCs were counted and calculated as cells/ml [26]. For haematocrit (Hct) or pack cell volume, microcapillary tubes were third-filled with bloods. The other ends of the tubes were sealed and the tubes were then centrifuged at 11,500 rpm for 4 min [27]. A percentage of Hct was read using microhaematocrit reader. Concentrations of haemoglobin (Hb) were assessed by thoroughly mixing blood samples with Drabkin's solution and incubating for 10 min for the conversion of Hb to cyanomethaemoglobin. The transmittance was recorded at 540 nm using a spectrophotometer [28]. The contents (g/dl) of Hb were calculated by comparing with a standard Hb.

Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the formulae as follows [29]:

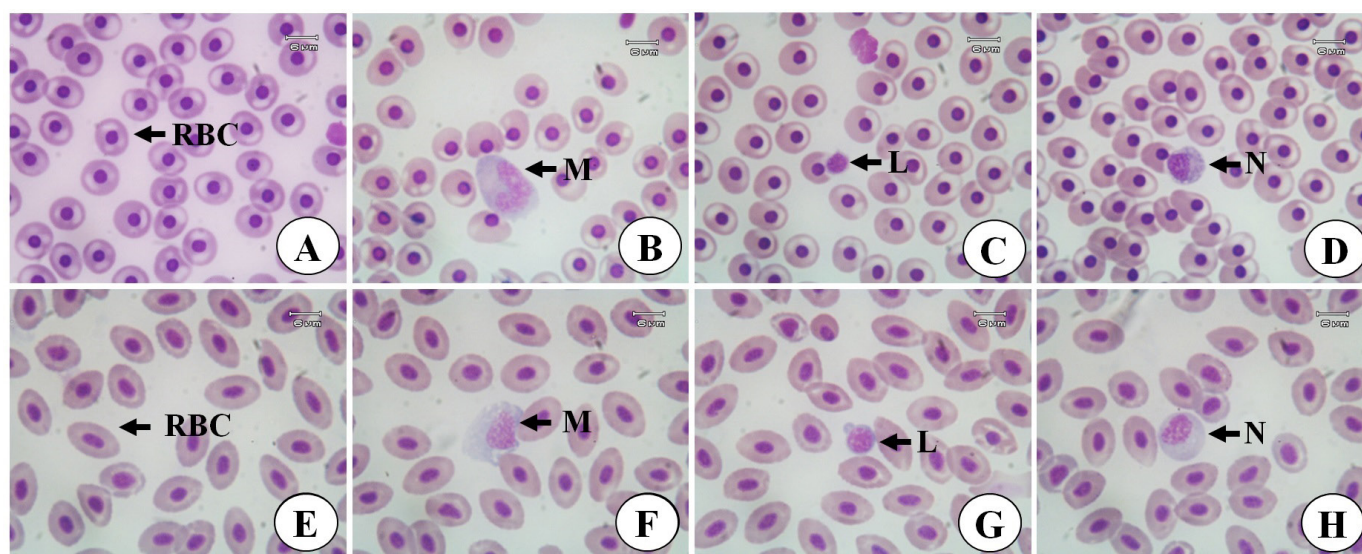$$\text{MCV (fl)} = [\text{Hct (\%)} \times 10] / \text{RBC} \qquad (1)$$
$$\text{MCH (pg)} = [\text{Hb (g/dl)} \times 10] / \text{RBC} \qquad (2)$$
$$\text{MCHC (g/dl)} = [\text{Hb (g/dl)} \times 100 / \text{Hb (\%)}] \qquad (3)$$

For investigating differential white blood cell counts, a drop of a blood sample from each fish was smeared on a cleaned microscopic slide. Three slides per fish were performed. All slides were dried at room temperature. All dried slides were fixed in absolute methanol for 10 min and stained with Giemsa-Wright stain. A total of 200 leukocytes per slide were counted to analyse the relative numbers of the blood cell types including lymphocyte, neutrophil, monocyte, eosinophil and basophil [30].

Investigations of micronucleus

To investigate micronucleus in peripheral bloods, a total of three smear slides were prepared as described above. A total of 5,000 erythrocytes per slide were examined to classify micronuclei,

Figure 1 Microscopic photographs of blood smears of hybrid catfish (A-D) and Nile tilapia (E-H). RBC (red blood cell), monocyte (M), lymphocyte (L) and neutrophil (N). Giemsa-Wright stain, 1,000x.

immature erythrocyte and erythrocytic nuclear abnormalities under an Olympus BX41 light microscope [31]. Chromatic bodies with small, circular or oval patterns indicated the occurrences of micronucleus. The presences of binuclei, blebbed nuclei, lobed nuclei, kidney-shape nuclei and notched nuclei were considered as erythrocytic nuclear abnormalities [32]. The percentages of micronuclei, immature erythrocyte and nuclear abnormal found in each fish species were calculated.

Histological examinations

The hepatic and renal tissues of fishes were fixed in Bouin's solution. They were dehydrated through the increasing grades of alcohol and cleared in xylene. The 6-μm thick sections were prepared after embedding. All sections were stained with haematoxylin and eosin (H & E) and permanently mounted. The stained slides of livers and kidneys of hybrid catfish and Nile tilapia were comparatively examined for their histoarchitecture. Histological changes in the permanent slides were also examined using semiquantitatively scoring methods [33]. The degrees of alterations were scored ranging from - to +++ depending on the severities. No occurrence of histological changes (-), less than 30 % (+), 30 - 70 % (++) and more than 70 % of occurrence. Micrographs of livers and kidneys tissues were taken using a light microscope (Olympus BX41) coupled with a camera.

RESULTS

Haematological parameters

Table 1 summarizes haematological profiles of hybrid catfish and Nile tilapia. We found that haematological values including Hb, Hct, MCV and MCH of Nile tilapia were higher than those in hybrid catfish. In contrast, RBCs, MCHC and WBCs in hybrid catfish was more than in Nile tilapia. Microscopic examinations in blood smears found four types of leucocyte and red blood cell or erythrocyte. Morphology of

Table 1 Hematological parameters of hybrid catfish and Nile tilapia.

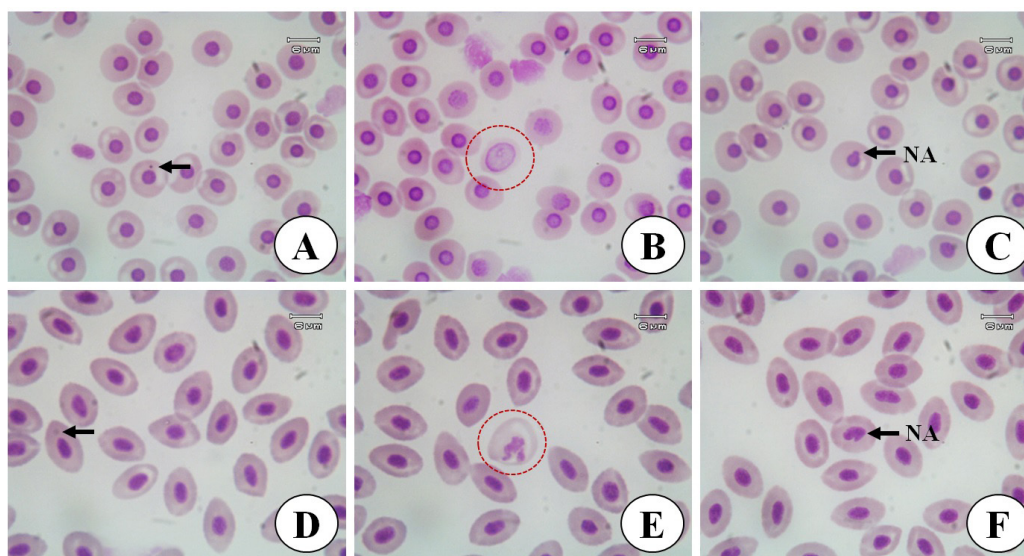| Haematological indices	Fish species	
	Hybrid catfish	Nile tilapia
RBCs (x 10^9 cells/ml)	3.4 ± 0.6	2.0 ± 0.7
Haematocrit (%)	36.0 ± 6.9	51.0 ± 7.5
Haemoglobin (g/dl)	8.8 ± 1.2	11.4 ± 1.7
MCV (fl)	103.0 ± 3.7	224.0 ± 50.0
MCH (pg)	27.0 ± 3.9	49.0 ± 1.8
MCHC (g/dl)	25.0 ± 5.1	22.7 ± 2.1
WBCs (x10^6 cells/ml)	19.0 ± 1.6	8.0 ± 2.1
Neutrophil (%)	72.0 ± 8.9	56.0 ± 9.7
Lymphocyte (%)	14.0 ± 8.6	21.0 ± 9.1
Monocyte (%)	12.0 ± 5.8	23.0 ± 5.6
Eosinophil (%)	0.0 ± 0.0	0.6 ± 0.6
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0

Values are expressed as mean ± S.D. of ten fishes each.

erythrocyte and leucocytes are shown in Fig. 1. The mature RBCs were the predominant cell types found in blood smears of both species. However, RBC size of hybrid catfish seem to smaller than in Nile tilapia. Catfish RBC were spherical cell and nucleus while Nile tilapia had ellipsoidal cell with oval nucleus. For differential count, four types of leucocytes including neutrophil, monocyte, lymphocyte and eosinophil were found. Among them, neutrophils followed by lymphocyte were the most frequent cells recorded in both fish species. High percentages of all types of WBC were recorded in Nile tilapia, except for neutrophil. A few of eosinophil was found in Nile tilapia. On the other hand, eosinophil and basophil were not observed in hybrid catfish.

Micronucleus indices

Fig. 2 illustrates details of various abnormalities in mature RBC of fishes. The small circular or oval particles with variable sizes were

Figure 2 Microscopic photographs of micronucleus in RBC of hybrid catfish (A-C) and Nile tilapia (D-F). Micronuclei (dark arrow), immature erythrocyte (circle) and nuclear abnormalities (NA). Giemsa-Wright stain, 1,000x.

Table 2 Frequency of micronuclei, immature erythrocyte and nuclear abnormality in peripheral blood erythrocytes of hybrid catfish and Nile tilapia.
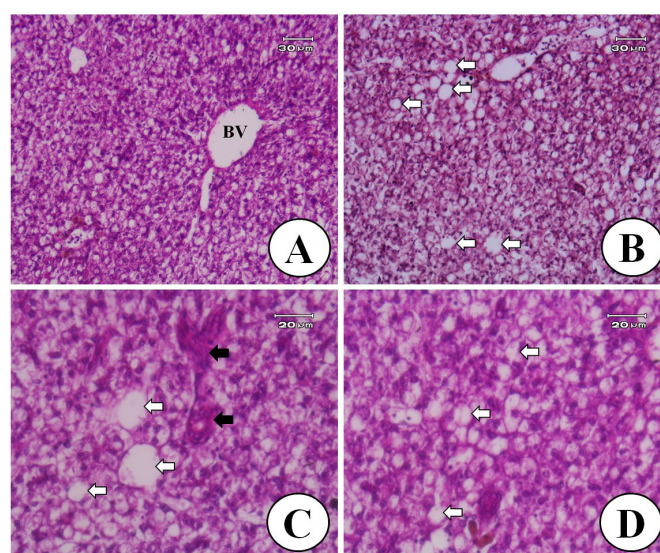
Micronucleus	Fish species	
	Hybrid catfish	Nile tilapia
Micronuclei (%)	0.05 ± 0.05	0.08 ± 0.06
Immature RBC %)	1.14 ± 0.06	0.60 ± 0.48
Abnormal nuclei (%)	0.32 ± 0.16	1.13 ± 0.48

Values are expressed as mean ± SD of ten fishes each.

noted in both hybrid catfish and Nile tilapia. The micronuclei was located near or far to the main nucleus in the periphery of the cells. The presences of the larger cell than the mature RBC with irregular shapes of the nucleus indicate immature erythrocytes. A nuclear abnormal, lobed nuclei was observed in hybrid catfish (Fig. 2 (C)) while kidney-shaped nuclei were found in peripheral bloods of Nile tilapia (Fig. 2 (F)). Table 2 displays the numbers of micronucleus in RBC. It was found that the high frequencies of micronuclei, immature erythrocyte and erythrocytic nuclear abnormality were noted in Nile tilapia when compared to catfish.
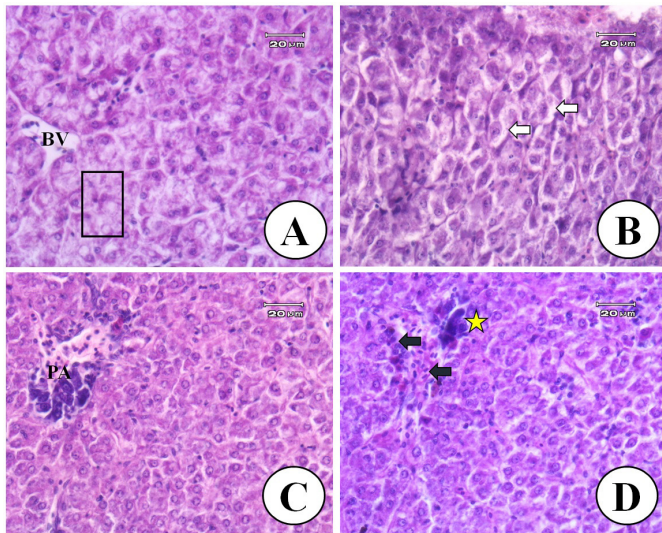
Histological examinations

Histological observations found that hepatic tissues of both fish species consisted of hepatic cords with a simple layer of hepatocytes. Hepatocytes were polyhedral with a rounded nuclei. Hepatic cords were separated by irregular shapes of hepatic sinusoids. The sinusoidal tract were converged to the blood vessels of central lobular vein. In addition, pancreatic tissues was embedded in the liver Nile tilapia. Histopathologically, alterations in liver tissues of hybrid catfish were more severe than in Nile tilapia [Table 3]. Vacuolated hepatocytes with irregular arrangements of hepatic cords were markedly seen in catfish's liver [Fig. 3]. In liver tissues of Nile tilapia, blood congestion in their hepatic sinusoids, aggregations of Kupffer cells, absence of hepatic nuclei, necrotic cells and vacuolization were slightly seen [Fig. 4].

Figure 3 Histopathological photomicrographs show hepatic tissues of hybrid catfish (A-D). Blood vessel (BV) was seen in central area of hepatic lobule (A). Vacuolization (white arrow) was markedly found (B-D), while blood congestion (dark arrow) was slightly seen (C). H&E stain, 200x and 400x.

In renal tissues, histoarchitecture in all fishes consisted of nephron with glomeruli and Bowman's capsule and renal tubule with different sizes. Most of tubular epithelia were cuboidal cells. A large area of haemapoietic tissues were seen. Histopatological observations showed that severe alterations were found in renal tissues of hybrid catfish when compared to Nile tilapia. Histological changes mildly found in both fish species were shrunken glomerulus, detachment of tubular epithelium, leukocyte infiltration and blood congestions.

Figure 4 Photomicrographs illustrate hepatic tissues of Nile tilapia (A-D). They contained blood vessel (BV) and pancreatic area (PA) (A and C). Histopathological indices such as absence of nucleus (square), vacuolization (white arrow) as well as leucocyte infiltration (dark arrow) and necrotic area (yellow star) were found in A, B and C, respectively. H&E stain, 400x.

Table 3 Histopathological alterations in livers of hybrid catfish and Nile tilapia.

Histological changes	Fish species	
	Hybrid catfish	Nile tilapia
Blood congestion	+	+++
Haemorrhage	+	++
Vacuolization	+++	+
Pyknotic cell	++	+
Leucocyte infiltration	++	++

+ (mild), ++ (moderate), +++ (severe)

Table 4 Histopathological alterations in kidneys of hybrid catfish and Nile tilapia.

Histological changes	Fish species	
	Hybrid catfish	Nile tilapia
Blood congestion	+	+
Shrunken glomeruli	+	+
Detached tubular cells	++	+++

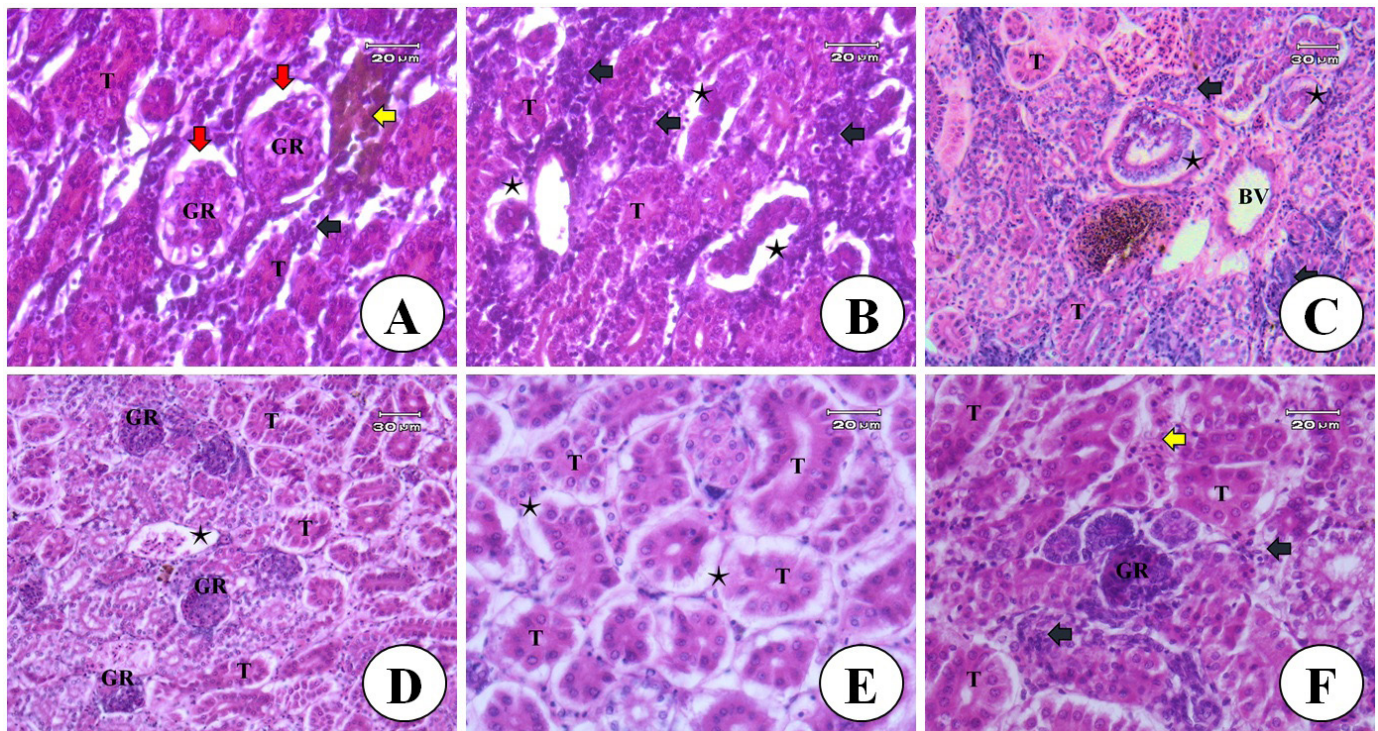+ (mild), ++ (moderate), +++ (severe)

DISCUSSION

Blood parameters in fish can significantly change in response to stress conditions [6-10]. Thus, studies of haematological indices can determine physiological responses and health status of fishes in different areas. In addition, studies of pathological changes together with blood parameters can provide the knowledge for the identification of conditions that cause stress or mortality in fisheries. In this study, the parameters of erythrocytes including Hct, Hb, MCV and MCH in Nile tilapia were higher than those in hybrid catfish. In contrast, the highest frequencies of RBCs, MCHC and WBCs were noted in hybrid catfish. This indicates that hybrid catfish required more of oxygen uptake than Nile tilapia in the stress conditions caused by hypoxia [34]. It is possible that they can survive under low oxygen levels by either elevating ventilation rates in acute hypoxic condition or increasing rates of erythropoiesis in chronic hypoxia [35]. On the other hand, hybrid catfish can tolerate and adapt themselves to survive in hypoxic environments by changing their ventilations, oxygen-sensitive chemoreceptors and also autonomic control of the heart as evidenced in channel catfish [36].

The results of haematology from our study were similar to a previous study which showed that higher numbers of RBCs were found in a variety of the *Claris* genus than in *O. niloticus* [37]. Interestingly, catfish had WBCs and neutrophil more than in Nile tilapia. On the other hand, the most frequent of lymphocyte and monocyte were found in Nile tilapia. These indicate signs of inflammations which are the immune responses of fishes against infections. Basophil was not observed in either fishes, while few eosinophils were noted in Nile tilapia. This confirms the reports on different teleosts in which basophil was not recorded in plaice, rainbow, salmon pink and brown trout. Other studies revealed that *O. niloticus* exposed to formalin at 1.56 mg/l suffered from hypoxia [38] and cement powder at 1.23-19.60 mg/l also produced haematological changes in this fish species [39]. Interestingly, acute exposures to hazardous agents such as chlopyrifos and mercury at low concentrations did not alter any haematological parameters in *O. niloticus* [10, 40]. Therefore, we suggest that the haematological parameters of hybrid catfish and Nile tilapia investigated in this study were in reference ranges when compared to healthy fishes [37]. Moreover, variations in their blood parameters may be the results of the homeostasis and adaptation to their environments.

Micronuclei are extra-nuclear bodies which are the result of fragmented chromosomes during cell division. They are found in cytoplasmic periphery near or far from the main nucleus. Frequencies of micronuclei in mature RBC of fish can be used as an indicator for detecting a number of mutagenic agents [41]. Normally, immature erythrocytes are found only in hematopoietic organs. Thereby, an occurrence of immature erythrocytes in peripheral blood indicates the physiological responses of fish against stresses. This study revealed that Nile tilapia had higher frequencies of micronuclei, immature erythrocytes and nuclear abnormalities in peripheral blood than in hybrid catfish. These results confirm that Nile tilapia are more sensitive than hybrid catfish. However, the numbers of nuclear abnormalities from our investigation were lower than in the previous report [42]. This implies that no pollutants or stressors in their habitats produced genetic damage in either fishes. In contrast to our study, cyclophosphamide and mitomycin C significantly increased the frequencies of micronuclei in three fish species-*Tilapia rendalli*, *O. niloticus* and *Cyprinus carpio* [43]. Additionally, micronuclei and blebbed and lobed nuclei in erythrocytes were clearly observed in Nile tilapia exposed to petroleum oils and chromium [42]. Immature erythrocytes observed in a wide range of teleosts demonstrate hypoxic conditions. This is likely due to the fishes having released immature

Figure 5 Histopathological photomicrographs of renal tissues of hybrid catfish (A-B) and Nile tilapia (C-F) show shrunken glomeruli (red arrow), detachment of tubular epithelium (star) and blood congestion (yellow arrow). H&E stain, 200x and 400x. Glomerulus (GR), renal tubule (T), blood vessel (BV) and leukocyte infiltration (dark arrow).

erythrocytes from their hematopoietic sites into their peripheral blood for enhancing oxygen transport.

The liver is the largest organ in the body associated with digestion and metabolism as well as protein synthesis, storage, bile secretion and detoxification. Therefore, injuries to hepatic tissues can induce liver dysfunctions. In this study, both hybrid catfish and Nile tilapia revealed histological alterations in their hepatic tissues. Blood congestion, leucocyte infiltration, vacuolization and pyknotic cells were seen in both fishes. Blood congestion in hepatic sinusoids demonstrates hyperemia secondary to the inflammations. The absence of hepatic nuclei, pyknotic cells and vacuolization are likely due to hepatocyte damage induced by harmful contaminants. Likewise, Nile tilapia treated with copper and endosulfan, an herbicide, displayed vacuolization, melanomacrophage aggregation, cell necrosis and edema in hepatic tissues [44].

The kidney is important in the excretory system and in haematopoiesis of fishes. The detachment of tubular epithelium as well as shrunken glomeruli and mononuclear infiltrations observed in renal tissues of both fish species indicate the reductions in renal function. This implies that contaminations of pollutants in blood circulation alter cellular functions and degrade renal capacity in excretion. Leukocyte infiltrations also reflect the immune response of fish against contaminants. Previously, hypertrophy of renal tubules, edema, blood congestion and glomerulonephritis were seen in Nile tilapia treated with 5 mg/l of ammonia [45]. Furthermore, the pesticide chlorpyrifos also damaged kidney tissues of catfish (*Heteropneustes fossilis*) by producing shrinkage of glomerulus, vacuolization and tubular cell necrosis [46]. However, histopathological alterations in both Nile tilapia and hybrid catfish observed in this study were mild

inflammations when compared to the literature described above. These changes are likely due to the impact of the restricted environment with low oxygen levels more than the effects of hazardous agents. The results obtained from our study can provide basic data for application in research of environmental pollution.

CONCLUSION

Taken together, it can be concluded that Nile tilapia had higher levels of red blood cell indices as well as nuclear abnormalities than hybrid catfish. On the other hand, the levels of RBCs, WBCs and MCHC in hybrid catfish were higher than in Nile tilapia. A higher frequency of micronuclei, immature erythrocytes and abnormal nuclei was found in the peripheral blood of Nile tilapia. However, these parameters were normal according to reference standards. Although histopathological changes were observed in liver and kidney tissues of both fish species, the alterations were slight. We expect that eventually the changes in haematological values and histopathology of these two fishes can be used for monitoring the quality of river environments in further studies.

ACKNOWLEDGEMENTS

REFERENCES

1. A. Goksoyr, A. Husoy, H. Larsen, J. Klungsoyr, S. Wilhelmsen, A. Maage, E. Brevik, T. Andersson, M. Celander, M. Pesonen, L. Förlin, Environmental contaminants and biochemical responses in flatfish from the Hvaler Archipelago in Norway, *Arch. Environ. Contam. Toxicol.*, 1991, 21, 486-496.

2. U.U. Gabriel, G.N.O. Ezeri, O.O. Opabunmi, Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822), *Afr. J. Biotechnol.*, 2004, 3, 463-467.

3. A. Ivanc, E. Hasković, S. Jeremić, R. Dekić, Hematological evaluation of welfare and health of fish, *Praxis veterinaria*, 2005, 53, 191-202.

4. M. Tavares-Dias, F.R. Moraes, Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf.), with an assessment of morphological, cytochemical, and astructural features, *Vet. Clin. Pathol.*, 2007, 36, 49-54.

5. R.M.G. Wells, J. Baldwins, R.S. Seymour, K. Christian, T. Brittain, Red blood cell function and haematology in two tropical freshwater fishes from Australia, *Comp. Biochem. Physiol. A.*, 2005, 141, 87-93.

6. R. Ullah, A. Zuberi, M. Naeem, S. Ullah, Toxicity to hematology and morphology of liver, brain and gills during acute exposure of Mahseer (*Tor putitora*) to cypermethrin, *Int. J. Agric. Biol.*, 2015, 17, 199-204.

7. C. Jayaprakash, N. Shettu, Changes in the hematology of the freshwater fish, *Channa punctatus* (Bloch) exposed to the toxicity of deltamethrin, *J. Chem. Pharm. Res.*, 2013, 5, 178-183.

8. M. Kandari, J.V.S. Rauthan, Hematological changes in fish *Mastacembelus armatus* (Lacepede) of Song River, *Sch. Acad. J. Biosci.*, 2015, 3, 34-37.

9. L.L. Correa, L.A.D. Bastos, P.S. Ceccarelli, N.S. Dos Reis, Hematological and histopathological changes in *Hoplias malabaricus* from the Sao Francisco River, Brazil caused by larvae of *Contracaecum* sp. (Nematoda, Anisakidae), *Helminthologia.*, 2015, 52, 96-103.

10. N.M. Ishikawa, M.J.T.Ranzani-Paiva, J.V. Lombardi, C.M. Ferreira, Hematological parameters in Nile tilapia, *Oreochromis niloticus* exposed to sub-lethal concentration of mercury, *Braz. Arch. Biol. Technol.*, 2007, 50, 619-626.

11. A. Rodriguez-Cea, F. Ayllon, E. Garcia-Vazquez, Micronucleus test in freshwater fish species: an evaluation of its sensitivity for application in field surveys, *Ecotoxixol. Environ. Saf.*, 2003, 56, 442-448.

12. M. Pacheco, M.A. Santos, Induction of micronuclei and nuclear abnormalities in the erythrocytes of *Anguilla anguilla* l exposed either to cyclophophamide or to bleached Kraft pulp mill effluent Fresenius, *Environ. Bull.*, 1996, 5, 746-751.

13. G.M. Alink, E.M.H. Frederix-Walters, M.A. van der Gaag, J.F.J. van der Kerkhoff, C.L.M. Poels, Induction of sister-chromatid exchanges in fish exposed to Rhine water, *Mutat. Res.*, 1980, 78, 369-374.

14. C.K. Grisolia, C.M.T. Cordeiro, Variability in micronucleus induction with different mutagens applied to several species of fish, *Genet. Mol. Biol.*, 2000, 23, 235-239.

15. H.M. Cooley, J.F. Klaverkamp, Accumulation and distribution of dietary uranium in lake whitefish (*Coregonus clupeaformis*), *Aquat. Toxicol.*, 2000, 48, 375-392.

16. N.S. Loumbourdis, Hepatotoxic and nephrotoxic effects of cadmium in the frog *Rana ridibunda*, *Arch. Toxicol.*, 2005, 79, 434-440.

17. C. Fenoglio, E. Boncompagni, M. Fasola, C. Gandini, S. Comizzoli, G. Milanesi, S. Barni, Effects of environmental pollution on the liver parenchymal cells and kupffer-melanomacrophagic cells of the frog *Rana esculenta*, *Ecotoxicol. Environ. Saf.*, 2005, 60, 259-268.

18. P. Samanta, N. Bandyopadhyay, S. Pal, A.K. Mukherjee, A.R. Ghosh, Histopathological and ultramicroscopical changes in gill, liver and kidney of *Anabas testudineus* (Bloch) after chronic intoxication of almix (metsulfuron methyl 10.1%+chlorimuron ethyl 10.1%) herbicide, *Ecotoxicol. Environ. Saf.*, 2015, 122, 360-367.

19. W. Jiraungkoorskul, S. Sahaphong, N. Kangwanrangsan, Toxicity of copper in butterfish (*Poronotus triacanthus*): tissues accumulation and ultrastructural changes, *Environ. Toxicol.*, 2007, 22, 92-100.

20. S. Saenphet, W. Thaworn, K. Saenphet, Histopathological alterations of the gills, liver and kidney in *Anabas testudineus* (Bloch) fish living in an unused lignite mine, Li District, Lamphun Province, Thailand, *Southeast Asian J. Trop. Med. Public Health.*, 2009, 40, 1121-1126.

21. L. Giari, M. Manera, E. Simoni, B.S. Dezfuli, Cellular alterations in different organs of European sea bass *Dicentrarchus labrax* (L.) exposed to cadmium, *Chemosphere.*, 2007, 67, 1178-1181.

22. [22]S. Pal, E. Kokushi, J. Koyama, S. Uno, A.R. Ghosh, Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos, *J. Environ. Sci. Health, B.*, 2012, 47, 180-195.

23. A.K. Mishra, B. Mohanty, Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch), *Sci. Totol. Environ.,* 2009, 407, 5031-5038.

24. Y.B. Koca, S. Koca, S. Yildiz, B. GÜrcÜ, E. Osanç, O. Tunçbaş, G Aksoy, Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* (Pisces: Perciformes) in the Cine stream (Aydin/Turkey) with determination of water pollution, *Environ. Toxicol.*, 2006, 20, 560-571.

25. O. Kori-Siakpere, J.E.G. Ake, E. Idoge, Haematological characteristics of the African Snakehead, *Parachanna Obscura*, *Afr. J. Biotechnol.*, 2005, 4, 527-530.

26. J.V. Dacie, S.N. Lewis, *Practical Haematology*, seventh ed., Churchill Livingstone, Edinburg, 1991.

27. G. Tanyer, *Hematology and Laboratory*, Ayyildiz Printery Inc. Publications, Ankara, 1985.

28. S.A. Levinson, R.P. Macfat, *Clinical laboratory diagnosis,* Lea and Febiger, Philadelphia, 1981.

29. B.A. Brown, *Haematological principles and procedures*, third ed., Lea and Febiger, Philadelphia, 1980.

30. J.B. Mirale, *Laboratory Medicine Haematology*, sixth ed., The CV mosby Publishing, London, 1982, pp. 883.

31. F. Ayllón, E. Garcia-Vazquez, Micronuclei and other nuclear lesions as genotoxicity indicators in rainbow trout *Oncorhynchus mykiss*, *Ecotoxicol. Environ. Saf.*, 2001, 49, 221-225.

32. K.R. Carrasco, K.L. Tilbury, M.S. Myers, Assessment of the Piscine micronucleus test as an *in situ* biological indicator of chemical contaminant effects, *Can. J. Fish. Aquat. Sci*., 1990, 47, 2123-2136.

33. N. Pantung, K.G. Helander, H.F. Helander, V. Cheevaporn, Histopathological alterations of hybrid walking catfish (*Clarias macrocephalus* x *Clarias gariepinus*) in acute and subacute cadmium exposure, *Environment. Asia.*, 2008, 1, 22-27.

34. T.F.A. EL-Khaldi, Effect of different stress factors on some physiological parameters of Nile tilapia (*Oreochromis niloticus*), *Saudi. J. Biol. Sci*., 2010, 17, 241-246.

35. D.J. Randall, Gas exchange in fish, in: W.S. Hoar, D.J. Randal (Eds), Fish Physiology, Vol. IV, Academic Press, New York, 1970, pp. 253-287.

36. M.L. Burleson, A.L. Carlton, P.E. Silva, Cardioventilatiory effects of acclimatization to aquatic hypoxia in channel catfish, *Resp. Physiol. Neurobi*., 2002, 131, 223-232.

37. A.G.M. Chester, P.B. Alessandra, *Erythrocytes of the poikilotherms: A phylogenetic odyssey*, Arti Grafiche Italo Cernia S.r.l. Naples, Italy, 2006.

38. E. Omoregie, T.G. Eseyin, P.C. Ofojekwu, Chronic effects of formalin on erythrocyte counts and plasma glucose of Nile tilapia (*Oreochromis niloticus*), *Asian Fish. Sci*., 1994, 7, 1-6.

39. A.K. Mohammed, A.B. Sambo, Haematological assessment of the Nile Tilapia *Oreochromis niloticus* exposed to sublethal concentrations of Portland cement powder in solution, *Int. J. Zool.*

Res., 2008, 4, 48-52.

40. M.I.G. Perez, Effect of chlorpyrifos on the hematology and phagocytic activity of Nile Tilapia cells (*Oreochromis niloticus*). *Toxicol. Mech. Methods.*, 2006, 16, 495-499.

41. T.D.S. Souza, C.S. Fontanetti, Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent, *Mutat. Res*., 2006, 605, 87-93.

42. T. Cavas, S.E. Gozukara, Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents. *Aqua. Toxicol*., 2005, 74, 264-271.

43. C.K. Grisolia, F.L.R.M. Starling, Micronuclei monitoring of fishes from Lake Paranoa, under influence of sewage treatment plant discharges. *Mutat. Res*., 2001, 491, 39-44.

44. A.M. Coimbra, A. Figueiredo-Fernandes, M.A. Reis-Henriques, Nile tilapia (*Oreochromis niloticus*), liver morphology, CYP1A activity and thyroid hormones after endosulfan dietary exposure. *Pestic. Biochem. Physiol*., 2007, 89, 230-236.

45. A.C.K. Benil, G. Koksal, A. Ozkol, Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus*) effects on gill, liver and kidney histology. *Chemosphere*., 2008, 72, 1355-1358.

46. S.K. Srivastava, P.R. Tiwari, A.K. Srivastav, Effects of chlorpyrifos on the kidney of freshwater Catfish, Heteropneustes fossilis. Bull. Environ. *Contam. Toxicol*., 1990, 45, 748-751.