Hematological study to evaluate the *Cyprinus carpio* fish healthy in two areas of Baghdad city

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Abstract

The current research was conducted to evaluate the health status of common carp fish (*Cyprinus carpio*) which are sold in the local markets of Baghdad city (ALTaji, AL Rasheed sell market) to demonstrate its suitability for human consumption, where fish samples were taken over two months (January and February 2017) for hematological tests: count of micronuclei in erythrocyte, percentage of leukocytes types (Lymphocyte, Eosinophil, Neutrophil, Basophil, Monocyte and Thrombocyte). The results of the current study showed that the common carp fish from AL Rasheed sell market have better health compared to fish that was taken from ALTaji sell market and therefore its suitability for human consumption.

Keywords: *Cyprinus carpio*, Hematological tests, Micronucleus, Leukocytes type, Fish healthy

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1. Introduction

Fish is an economically important natural resource for its high nutritional value, in addition to the role of some species in the elimination of disease vectors and dependence on other species as biological indicators to detect water pollution (Coad, 2010; Francis et al., 2014). The hematological analysis are important for assessing the health and physiological status of fish (Blaxhall, 1972; Hrubec et al., 2000) it shows how fish are affected by their environment (Gabriel et al., 2004) where the increased in industrial and agricultural activity aspects has led to an increase in the level of pollutants in the aquatic environment (Xu et al., 2014) thus, the possibility of the adoption of hematological tests as an indicator for monitoring water pollution (Modra et al., 1998).

One of the most important blood tests adopted in fish for the detection of contaminants is the micronuclei test in erythrocytes, which is widely used as an indicator to identify the sensitivity of fish when exposed to a complex mixture of water pollutants (De Flora et al., 1993). It is an indicator of genetic damage that occurs during the cell life cycle (Bolognesi and Hayashi, 2011). As well as, examining and determining the ratios of fish leukocytes types as an indicator to investigate the health status and evaluation of the immune system of fish which, varies depending on fish species (Rowley et al., 1988) gender and season (Modra et al., 1998) methods of breeding and nutrition (Svobodova et al., 1998) and the fish health status (Rehulka, 1996). Therefore, the present study aimed to evaluate the health status of the common carp fish (*Cyprinus carpio*) belonging to the family Cyprinidae and its suitability for consumption, which are sold in the local markets of Baghdad city. By calculating the number of micronuclei in erythrocytes and calculating the percentage of leucocyte’s types.

2. Materials and Methods

2.1 Collection of fish samples:
Common carp fish were purchased from the local markets of Baghdad city (Al-Taji, Al-Rasheed sell markets) for the period from January to the end of February 2017, twice a month (second and fourth weeks) and then transported alive with special plastic containers to the laboratory for the required blood tests.

2.2 Micronuclei Test:

The peripheral blood was obtained from the caudal vein of each fish, then smeared immediately and the micronuclei test done according to (AL-Sabti and Metcalfe, 1995; Ayllon and Garcia – Vazquez, 2000) methods.

2.3 Calculating the percentage of leucocyte’s types:

The percentage of leucocyte’s types were determined and counting according to (Seiverd, 1964) method.

3. Results and Discussion

The present study recorded the appearance of micronuclei in erythrocytes of common carp fish (Fig. 1) that were purchased from Al Rasheed and AlTaji sell markets in Baghdad province(Table - 1, Table - 2).

Fig (1): Micronuclei in erythrocytes of common carp fish (*Cyprinus carpio*)

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Number of Fish</th>
<th>Mean of micronuclei ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>The second week of January</td>
<td>4</td>
<td>2 ± 0.557</td>
</tr>
<tr>
<td>The fourth week of January</td>
<td>3</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>The second week of February</td>
<td>4</td>
<td>6 ± 0.5</td>
</tr>
</tbody>
</table>
While Table (2) showed an increase in the average number of micronuclei in the erythrocytes of fish that was taken from Al Taji sell market in the second (4 ± 0) and fourth (5 ± 0.5) weeks of January 2017 and the second week (4 ± 0.5) of February 2017, meanwhile, the average number of micronuclei was decreased in the fourth week (1 ± 0) of February 2017.

**Table (2):** The mean of micronuclei in erythrocytes of *Cyprinus carpio* fish

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Number of fish</th>
<th>Mean of micronuclei ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second week of January</td>
<td>3</td>
<td>4 ± 0</td>
</tr>
<tr>
<td>Fourth week of January</td>
<td>4</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>Second week of February</td>
<td>5</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>Fourth week of February</td>
<td>3</td>
<td>1 ± 0</td>
</tr>
</tbody>
</table>

It was observed through the study of the results of micronuclei test in fish, a difference in their numbers according to the intensity of water pollution in which these fish are present, where the fish chromosomes are affected when exposed to physical effects such as radiation (Lloyd and Edwards, 1983) or chemical agents like heavy metals (Krishnaja and Rege, 1982) pesticides and toxins (AL-Sabti, 1991). The appearance of micronuclei is attributed to the effect of pollutants on the erythroblast, which is located in the liver, kidneys, and spleen, which represent the organs that responsible for the formation and production of red blood cells in fish (Nilsson and Holmgren, 1986). Thus, any damage that occurs in the erythroblast will be evident in the erythrocytes, specifically on the genetic material of these cells. The pollutants can affect chromosomes through break it in multiple regions, then leads to the formation of non-centromere pieces, which will retard as micronuclei in the cytoplasm (Çavaş and Ergene-Gözükara, 2003) or their effect on spindle fibers that responsible for pulling chromosomes towards the polar of the cell during mitosis division, then prevents the formation of chromosomes in some areas and one or more chromosomes fail to retreat with the rest of the chromosomes during anaphase and the chromosomes that were left in the cytoplasm become a micronuclei (AL-Sabti, 1991).

The observed number of micronuclei in erythrocytes in the bloodstream may be much lower than the number of newly formed erythrocytes, where the red blood cell in fish passes five or six divisions from its formation until it reaches the bloodstream (Nilsson and Holmgren, 1986) thus, the number of micronuclei decreases as a result of their loss by repeated divisions (Abramssson, 1997) or the percentage of mature red blood cells with micronuclei in the bloodstream may be lower than the percentage of erythroblast in which the chromosomal aberration occurred, it was reported that some of these damaged erythroblasts do not reach the bloodstream (Hoofman and Ratt, 1982). Thus, the loss of these micronuclei means loss of a part of the genetic material that in turn is responsible traits.
of organism; also, their appearance indicates a damage in the cell DNA, because of the similarity in molecular structure of genetic material (DNA) in different organisms, any pollutant that affects the structure of DNA in a particular species will also affect the genetic material of another different organism (AL-Sabti, 1985). Therefore, the pollutants whose toxicity affects fish may be more toxic to mammals including humans (Hooftman, 1981).

The study of the percentage of leucocyte’s types in common carp fish showed appearance of all types of leucocytes (Lymphocyte, Eosinophil, Neutrophil, Basophil, Monocyte) and thrombocyte within the blood smears of the fish samples that was taken from Al-Rashid and Al-Tajisell market during the study months (Figures - 2,3,4,5,6) as well as, the difference in the percentage of these types of cells and thrombocyte between the two study areas (table – 3 and table – 4).

Fig. (2): Basophil (long arrow) & Lymphocyte (short arrow)

Fig. (3): Eosinophil

Fig. (4): Neutrophil

Fig. (5): Monocyte

Fig. (6): Thrombocyte
showed an increase in the percentage of lymphocytes in the fish samples taken from Al-Rasheed sell market in the second week (6.1%) and fourth week (5.3%) of February compared with the second and fourth weeks (3.6%) of January, while there was an increase in their percentages in the second week (4.4%) and fourth week (4.8%) of January and second week (4.3%) of February compared to the fourth week (3.6%) of February in the fish samples taken from Al Taji sell market (table – 4).

The lymphocyte (Fig. 2) is a non-granular spherical cell with a large, dark, rounded nucleus that occupies most of the cell surrounded by a small amount of non-granular basal cytoplasm (Tripathi, 2014). It has been previously reported that high lymphocyte levels occur in fish living within highly polluted aquatic systems and poor or contaminated water quality (Hrubec et al., 2000) and high bacterial infection (Ali and Ansari, 2012; Ibrahim and AL-Khayat, 2017) this is shown by the percentage of lymphocytes observed in the current study, as carp fish taken from Al-Taji sell market that was more affected by the contaminated environment because the high rates of these cells in the most study weeks (Table – 4) compared with the fish taken from Al-Rashid sell market (Table – 3). Table 3 also showed an increase in the percentage of thrombocytes in the second week (6.1%) and the fourth week (5.2%) of February, as well as their percentage in the fish taken from Al-Taji sell market in the second (5.4%) and fourth week (5.1%) of January and the second week (5.1%) of February (Table – 4). Thrombocytes (Fig.6) is known to be oval, spindle shape plays an important role in the coagulation process as well as being responsible for controlling the amount of fluid flowing from the wound surface in fish (Campball and Ellis, 2007). As for the Eosinophil (Fig. 3), it is a cell with a cytoplasm containing large oval-shaped granules and a multi-lobed nucleus decentralized location (Tripathi, 2014) which, recorded the highest rate of appearance in the second week (2.5%) and the fourth week (2.8%) of January compared to the second (0.7%) and fourth (1%) weeks of February for carp fish from Al-Rasheed sell market (Table – 3) while their rates were low during January and February in the blood smears of the fish samples from Al-Taji sell market (Table – 4). Generally, the rates of appearance of this cell is normal within the fish of both study areas, where a previous study of carp fish infected with bacterial infections showed that the Eosinophil ratio ranged between (12 – 14 %) (Ali and Ansari, 2012). It has been pointed out that Eosinophil is involved in the inflammatory response because they have a specific gobbling ability and are generally less common in bonefish (Campbell, 2004).

Table (3): The Percentage of leukocytes types in *Cyprinus carpio* fish From AL-Rasheed sell market

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Lymphocyte</th>
<th>Eosinophil</th>
<th>Neutrophil</th>
<th>Basophil</th>
<th>Monocyte</th>
<th>Thrombocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>The second week of January-2017</td>
<td>3.6 ± 0.581</td>
<td>2.5 ± 1.707</td>
<td>0.9 ± 0.986</td>
<td>1.7±0.449</td>
<td>0.6 ± 0.255</td>
<td>3.3 ± 0.16</td>
</tr>
<tr>
<td>The fourth week of January-2017</td>
<td>3.6 ± 0.254</td>
<td>2.8 ± 0.277</td>
<td>1.4 ± 0.577</td>
<td>1.8±0.055</td>
<td>0.9 ± 1.527</td>
<td>4.2 ± 0.618</td>
</tr>
<tr>
<td>The second week of February-2017</td>
<td>6.1 ± 1.362</td>
<td>0.7 ± 1.154</td>
<td>2 ± 0.365</td>
<td>1.6 ± 1.315</td>
<td>1.4±1.081</td>
<td>6.1 ± 1.732</td>
</tr>
<tr>
<td>The fourth week of February-2017</td>
<td>5.3 ± 1.549</td>
<td>1 ± 1.849</td>
<td>1.6 ± 1.5</td>
<td>1.6 ± 0.947</td>
<td>1.1 ± 0.362</td>
<td>5.2 ± 1.416</td>
</tr>
</tbody>
</table>
Table (4): The Percentage of leukocytes types in *Cyprinus carpio* fish
From AL-Taji sell market

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Lymphocyte</th>
<th>Eosinophil</th>
<th>Neutrophil</th>
<th>Basophil</th>
<th>Monocyte</th>
<th>Thrombocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>The second week of January-2017</td>
<td>4.4 ± 0.041</td>
<td>0.4 ± 0.577</td>
<td>0.9 ± 1</td>
<td>1 ±0.422</td>
<td>2.1 ± 1</td>
<td>5.4 ± 0.509</td>
</tr>
<tr>
<td>The fourth week of January-2017</td>
<td>4.8 ± 0.696</td>
<td>0.6 ± 0.362</td>
<td>0.8 ± 0.5</td>
<td>0.8±0.577</td>
<td>1.9 ± 0.816</td>
<td>5.1 ± 0.986</td>
</tr>
<tr>
<td>The second week of February-2017</td>
<td>4.3 ± 1.787</td>
<td>0.5 ± 0.577</td>
<td>0.8 ± 0.5</td>
<td>1 ± 0.5</td>
<td>1.4±0.055</td>
<td>5.1 ± 1.577</td>
</tr>
<tr>
<td>The fourth week of February-2017</td>
<td>3.6 ± 1.547</td>
<td>1 ± 1.645</td>
<td>1.5 ± 1.309</td>
<td>1.3 ± 1.732</td>
<td>0.8 ± 1.516</td>
<td>3.7 ± 1.645</td>
</tr>
</tbody>
</table>

4. Conclusion

Our study also showed that the percentage of the neutrophil cell (Fig. 4), a cell with a polymorphic and lobular nucleus with a decentralized position and non-granular cytoplasm (Tripathi, 2014) considered natural in the fish of two areas study, because of their low percentage, where it was mentioned that the rise in the proportion of this type of cell associated with the occurrence of inflammatory processes (Ali and Ansari, 2012) as well as for the Monocyte (Fig.5) a cell with a large nucleus of decentralized location resembling a horseshoe or in the form of lobes and cytoplasm with homogeneous dark colored granules (Tripathi, 2014) where their percentage was low in January compared to samples taken in February from Al-Rasheed sell market (Table – 3) it was also noted an increase in their appearance in blood smears during January and the second week of February form Al-Taji sell market fish (Table – 4) it was reported that the increase in the proportion of monocytes occurs as a response to the presence of inflammation in bony fish (Roberts and Ellis, 2001). The basophil was also seen within the blood smears of the current study fish (Fig.2), it is a cell with a lobular nucleus and a cytoplasm with large, round, light-colored granules, and they are larger than the eosinophil but are fewer (Tripathi, 2014) and sometimes missing in some fish species (Campbell, 2004). Where the proportion is normal among the samples taken in the second week (0.6%) and the fourth week (0.9%) of January, while there was an increase of it percentage in the second (1.4%) and fourth week (1.1%) of February in the fish blood smear of Al-Rasheed sell market (Table – 3). Therefore, the samples were taken in the second week (2.1%) and fourth week (1.9%) of January and the second week (1.4%) of February for Al-Taji fish sell market (Table – 4). The basophil is a white blood cell with a defensive function that is not specialized; it responds to the presence of foreign substances in the body and migrates to different parts of the body to attack and destroy these foreign substances by cellular eating processes as response to the presence of inflammation (Ali and Ansari, 2012).

Based on the results of the micronuclei test and the calculation of the percentage of leukocytes types for the current study, the common carp fish brought from Al-Rasheed sell market is better health and therefore more favorable for human consumption compared to the common carp fish from Al-Taji sell market.
References


