

## Immunohistochemistry of PPAR $\alpha$ , PPAR $\gamma$ and IFN $\gamma$ in kidney tissues of african catfish (*clarias gariepinus*) at different seasons

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**How to cite this article:** Hussein et al. Immunohistochemistry of PPAR $\alpha$ , PPAR $\gamma$  and IFN $\gamma$  in Kidney Tissues of African Catfish (*Clarias gariepinus*) at Different Seasons. *Veterinary Medicine and Public Health Journal* 1(1);(2020): 22-29.

**DOI:** <https://doi.org/10.31559/vmph2020.1.1.5>

**Received Date:** 28/12/2019 **Accepted Date:** 23/1/2020

### Abstract

The peroxisome proliferator-activated receptors (PPARs) and interferon  $\gamma$  (IFN $\gamma$ ) have a vital role in lipid homeostasis and immune functions respectively, and little is known about their beneficial role in kidney function during different seasons in catfish. We used immunohistochemistry for detection of cellular expression of PPAR $\alpha$ , PPAR $\gamma$ , and IFN $\gamma$  in the head kidney and posterior kidney of African catfish, we found that the PPAR $\alpha$  and PPAR $\gamma$  in head kidney gave a positive reaction in melanomacrophage centers (MMC), large lymphocytes, red blood cells, endothelial cells, and connective tissue capsule. This positive reaction reached its highest at winter. While in the posterior kidney they were detected in the cytoplasm of epithelium lining renal tubules and the summer was the highest season in expression. However, IFN $\gamma$  showed a positive reaction in the MMC and T-lymphocytes of the head kidney, which increased during the autumn season more than winter, spring and summer seasons. While in the posterior kidney its positive reaction was found in MMC, epithelial cells lining distal convoluted tubules and few in proximal convoluted tubules. The highest positive reaction in the posterior kidney was during the autumn season and was lowest in the summer season. This study established an essential role for both PPARs and IFN $\gamma$  in immune and excretory functions of the kidney as well as, a reverse relationship between PPARs and IFN $\gamma$  in the head and posterior kidneys of catfish.

**Keywords:** African catfish; PPARs; IFN $\gamma$ ; immunohistochemistry; kidney.

### 1. Introduction

The kidney of teleosts is divided into an anterior pronephric head kidney (HK) and a posterior mesonephric or trunk kidney. The head kidney is a vital lympho-myeloid or hematopoietic organ in fish which corresponds to bone marrow in higher vertebrates. The head kidney is located extra-peritoneally, while the posterior kidney is intra-peritoneally beside the vertebral column (Sailendri and Muthukkaruppan 1975; Ellis 1977; Fänge 1986; Meseguer et al. 1994; Press and Evensen 1999; Fijan 2002; Zapata et al. 2006; Kumar et al. 2016).

Peroxisome proliferation is mediated by nuclear receptors, peroxisome proliferator-activated

receptors (PPARs). Three subtypes of peroxisome proliferation have been found in many kinds of fish species (PPAR $\alpha$ , PPAR $\beta$ , and PPAR $\gamma$ ) (Ibabe et al. 2004). PPAR $\alpha$  is responsible for homeostasis of lipids as it controls gene expression and enzymes that regulate lipid metabolism (Kono et al. 2009; Cho et al. 2012). The PPAR $\alpha$ 1 cDNA was cloned from the liver tissue of yellow catfish (Zheng et al. 2015). PPAR $\alpha$  not only expressed in the liver but also in the kidney, intestine, muscles, and brain (Sanguino et al. 2004; Ibabe et al. 2005; Cho et al. 2012; Zheng et al. 2015). The peroxisomal  $\beta$ -oxidation enzymes usually activated under the influence of peroxisome proliferators (Cancio and Cajaraville 2000). PPAR $\gamma$  performs many functions in the kidney such as enhancement of the renin-



angiotensin system, so it regulates sodium and water resorption through epithelial lining renal tubules. As well as it controls lipid metabolism (KissTóth and Rószter 2008). IFN $\gamma$  is one of the cytokines involved in pro-inflammatory response in conjunction with tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 1 $\beta$  (IL-1 $\beta$ ) (Grayfer and Belosevic 2012). IFN $\gamma$  produced by T-cells (Farrar and Schreiber 1993). It involved in both cell-mediated and innate immune responses (Boehm et al. 1997; Schoenborn and Wilson 2007). It acts on macrophages to activate them for the production of both oxygen and nitrogen intermediates which are toxic agents for bacteria (Schroder et al. 2004; Zou et al. 2005; Yang et al.

2013). As well as, it has antiviral activity via stimulation of infected cells to produce several kinds of antiviral proteins and activation of T-cells functions (Schroder et al. 2004). Group II IFNs have been identified in cyprinids, salmonids, and turbot (Zou et al. 2007; Aggad et al. 2009; Sun et al. 2009).

In carp, the role of IFN- $\gamma$  in immune function was exerted via activation of antimicrobial property of both macrophages and neutrophils (Pijanowski et al. 2015).

This study aimed to show the cellular distribution of PPAR $\alpha$ , PPAR $\gamma$ , and IFN $\gamma$  in the head and posterior kidneys of African catfish during different seasons.

**Table (1): the number of fishes used in experiments during each season.**

Season	Winter (January)	Spring (April)	Summer (August)	Autumn (October)
No. of fishes	5	5	5	5

**Table (2): Summarize the effect of seasonal changes in PPAR $\alpha$ , PPAR $\gamma$  and IFN $\gamma$  expression in the anterior and posterior kidney of the African catfish according to morphometric analysis.**

Kind of tissue	PPAR $\alpha$				PPAR $\gamma$				IFN $\gamma$			
	Winter	spring	summer	autumn	Winter	spring	summer	autumn	Winter	spring	summer	autumn
<b>Anterior Kidney</b>												
MMCs	+++	++	±	++	+++	++	±	+	++	++	±	++
Lymphocytes	±	±	+	±	++	±	++	±	+++	+	+++	++++
C.T. Capsule	+	+	++++	+	+	+	++++	+	+++	+	—	++++
<b>Posterior Kidney</b>												
Renal tubules	+	+	++++	++	++	±	+	±	++++	+++	+	++++
MMCs	+++	+	+++	+++	+++	+	+++	+	++++	++++	±	+++
C.T. Capsule	+	+	++++	+	+	+	+++	+	+++	—	++++	

Very high positive (++++), high (+++), moderate (++), low (+), fairly positive (±) and negative (—). Melanomacrophage centers (MMCs) and connective tissue (C.T.).

## 2. Materials and Methods

### Fish sampling

Twenty adult African catfish (*Clarias gariepinus*) were used in the experiments as shown in the Table 1. The fishes were collected from two fish farms in Egypt. The samples were collected from the head kidney and posterior kidney of catfish.

### Histology

The samples were taken from fishes at various seasons and preserved in 10% neutral buffered formalin for one week, and then dehydrated, cleared and embedded inside paraffin wax. Serial sectioning by microtome at 5 $\mu$ m was obtained and stained by Hematoxylin and eosin stain for further histological observation. The histological techniques were according to (Hussein and Cao 2019).

### Immunohistochemistry

The samples were taken from fishes at various seasons and rapidly preserved in 4% paraformaldehyde for 24 hours then dehydrated, cleared and embedded in paraffin wax. Serial sections 5 $\mu$ m thick were cut and collected with positive charge slides. the immunohistochemical procedures were in accordance with (Hussein et al. 2020).

The sections were deparaffinized with xylene and hydrated with downgrades of ethanol, then

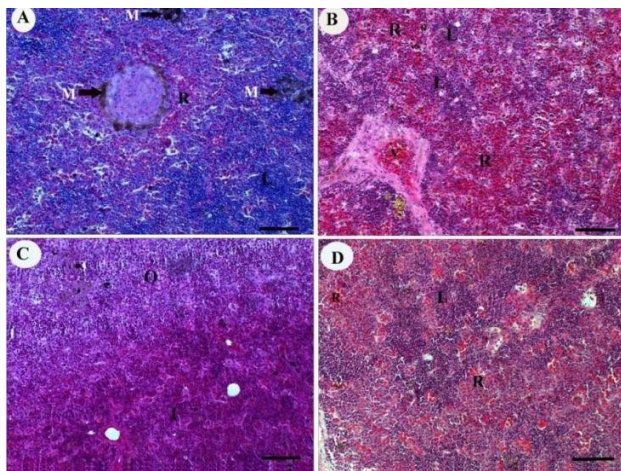
washed by phosphate-buffer saline (PH 7.4) for 15 minutes, then the slides were immersed in 3 % H2O2 in PBS (PH 7.4) for 30 min at room temperature and followed by triple washes by phosphate-buffer saline (PBS) and then blocked with blocking serum for 1 hour at room temperature. Then the sections were incubated at 4 °C overnight with the following antibodies:

- Rabbit polyclonal anti-PPAR alpha antibody
- Rabbit polyclonal anti-PPAR gamma antibody
- rabbit polyclonal anti-IFN $\gamma$

Antibodies were diluted by 1\500 in goat serum. The slides were washed with PBS three times.

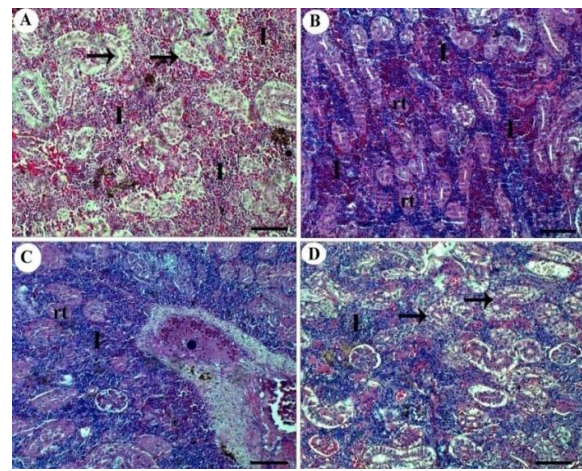
Then slides were incubated with biotinylated secondary goat anti-rabbit IgG antibodies and then were incubated with streptavidin-biotin-horseradish peroxidase complex for 30 min at room temperature. The sections were rewashed with PBS and immersed in diaminobenzidine (DAB) solution. Then the sections were counterstained with hematoxylin. Then the sections were dehydrated with serial grades of alcohol. Negative control slides were performed by using the same protocol but replacing the primary antibody with the bovine blocking solution. The sections were cleared in xylene and then mounted with DPX. A Zeiss light microscope was used for taking photos. The

immunohistochemistry findings were performed by morphometry analysis (Ibabe et al. 2005), to show seasonal changes in PPAR $\alpha$ , PPAR $\gamma$  and IFN $\gamma$



**Figure 1: Genral histological observations of head kidney of catfish during different seasons. (A) during winter season. (B) During spring season. (C) During summer season. (D) During autumn season. Lymphopoietic tissue (L), haematopoietic tissue (R), melanomacrophage centers (M), Posterior cardinal vein (v), outer zone (O) and inner zone (I). H&E stain. Scale bar indicated 100 $\mu$ m.**

expression in the anterior and posterior kidney of the African catfish as shown in the Table 2.



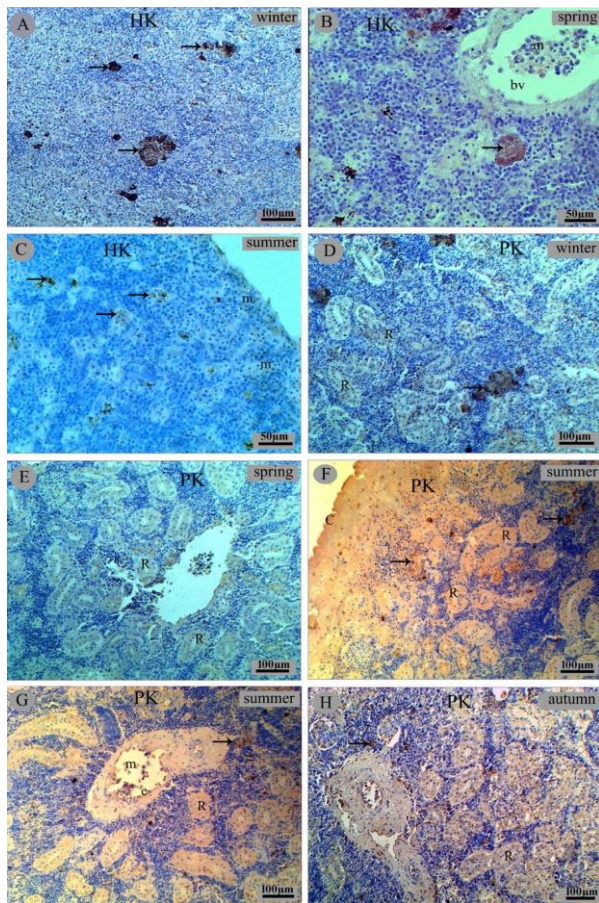
**Figure 2: General histological observations of posterior kidneys of catfish during different seasons. (A) During winter, (B) during spring, (C) during summer season and (D) during autumn season. Lightly stained epithelial lining renal tubules (arrows), interstitial lymphopoietic and haematopoietic tissue (I), normal darkly stained renal tubules (rt). H&E stain. Scale bar indicated 100 $\mu$ m.**

### 3. Results and Discussion

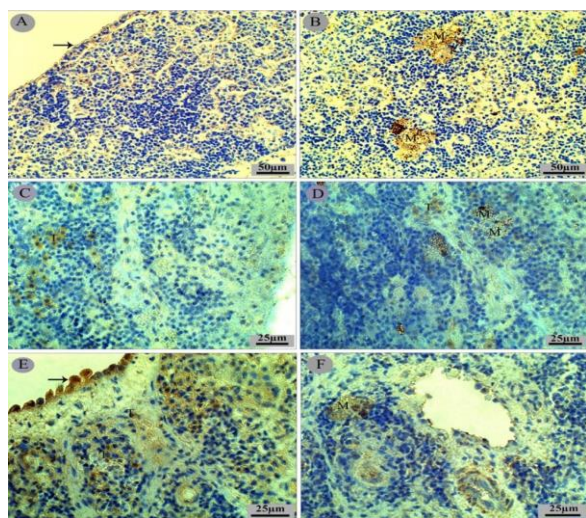
The histological observation of the head kidney revealed that it was formed from several lymphopoietic foci and among them, hematopoietic cells were dispersed. There were melanomacrophage centers which were a group of melanin-containing macrophages that present singly or in groups. Some interrenal tissue and chromaffin cells were observed around the central post cardinal vein (PCV). During the winter season, there was an increase in melanomacrophage centers; the lymphopoietic tissue was higher than hematopoietic tissue (Figure 1A). During the spring season, there was a noticeable increase in red blood cells in hematopoietic tissue which reached its maximum during this season and a decrease in melanomacrophage centers in comparison to winter season was noticed (Figure 1B). During the summer season, it was noticed that the parenchyma divided into outer small and light zone and inner dark and large zone, this shape was more evident in the summer season than other seasons. The head kidney was formed mainly from lymphopoietic cells which reached its maximum level during this season (Figure 1C). During the autumn season, both lymphopoietic and hematopoietic tissue began to decrease at this season in comparison to the summer season (Figure 1D). Our results were in accordance with (Attia et al.

2010) in thymus of *Tilapia nilotica* fish.

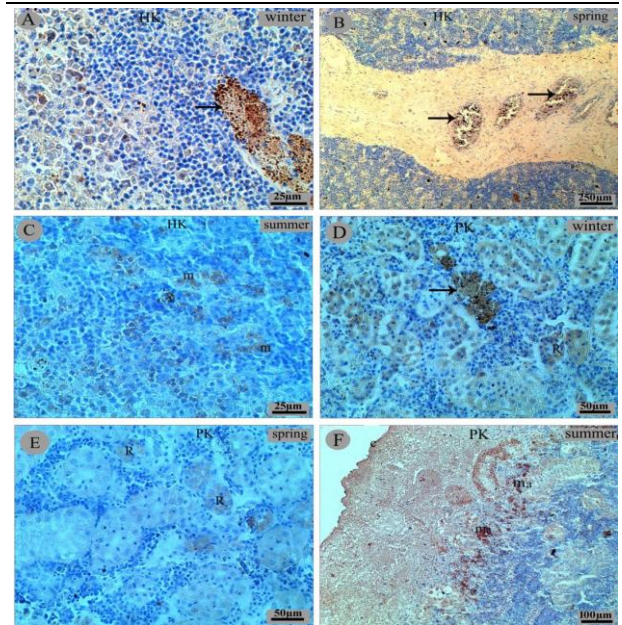
The hind kidney characterized by the presence of lymphopoietic and hematopoietic tissues in between renal tubules. During the winter season, there was obvious lightly staining property in epithelial lining proximal convoluted tubules, an increase in melanomacrophage centers and a decrease in both hematopoietic and lymphopoietic tissue (Figure 2A). During the spring season, there was a noticeable increase in both hematopoietic and lymphopoietic tissue in comparison with winter season and normal intact darkly stained epithelium of renal tubules was observed (Figure 2B). During the summer season more evident increase in lymphopoietic tissue than other seasons (Figure 2C). During the autumn season, there was a reappearance of lightly staining property in epithelium lining renal tubules with a decrease in both hematopoietic and lymphopoietic tissues (Figure 2D). We can conclude that low temperatures during winter and autumn diversely affect immune function in fish. The PPAR $\alpha$  showed a positive reaction in MMC, large lymphocytes and some red blood cells in head and posterior kidneys. As well as we showed a positive reaction in the cytoplasm of epithelial lining renal tubules of the posterior kidney (Figure 3).



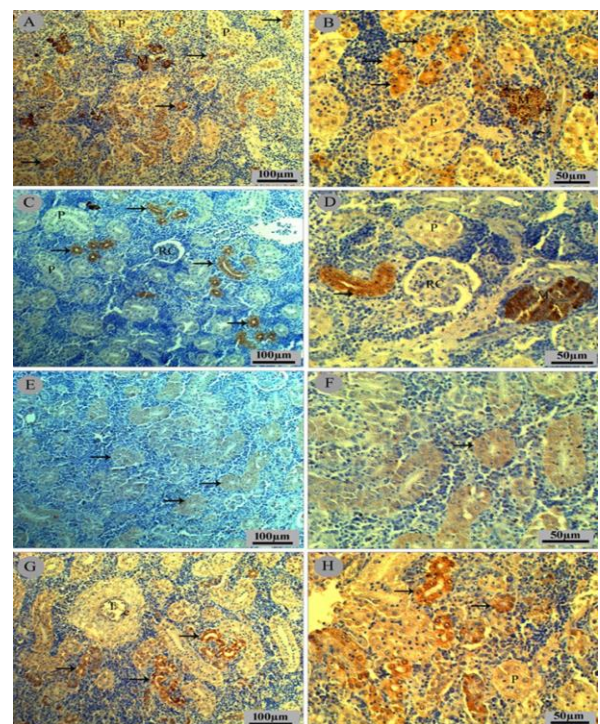
**Figure 3:** PPAR $\alpha$  immunohistochemistry in African catfish during different seasons. Head kidney of catfish (HK), and posterior kidney (PK). Melanomacrophage centers (arrows), large lymphocytes (m), blood vessels (bv), renal tubules (R), and endothelial cells (e).



**Figure 5:** The IFN $\gamma$  immunohistochemistry in the Head kidney of African catfish; (A) and (B) at winter season, (C) and (D) at summer season and (E) and (F) at the autumn season. Connective tissue capsule (arrow), melanomacrophage centers (M) and T- lymphocytes (T).



**Figure 4:** PPAR $\gamma$  immunohistochemistry in African catfish during different seasons. Head kidney of catfish (HK) and posterior kidney (PK). Melanomacrophage centers (arrows), large lymphocytes (m), macrophage (ma), renal tubules (R).



**Figure 6:** The IFN $\gamma$  immunohistochemistry in the posterior kidney of African catfish; (A) and (B) at winter season, (C) and (D) at spring season, (E) and (F) at summer season and (G) and (H) at the autumn season. Proximal convoluted tubules (P), distal convoluted tubules (arrows), melanomacrophage centers (M), renal corpuscles (RC), endothelial cells lining blood vessels (e) and T- lymphocytes (T).

PPAR $\alpha$  expression was affected by seasonal changes; (1) in head kidney, at winter there was high positive reaction of MMC (Figure 3A), at spring there was moderate positive reaction of MMC (Figure 3B) also moderate positive reaction of MMC was observed at autumn, while at summer there was shallow positive reaction of MMC and a low positive reaction of large lymphocytes especially under the capsule (Figure 3C). (2) Posterior kidney, in winter there was a high positive reaction in MMC and a low positive reaction in epithelial lining renal tubules (Figure 3D), in spring there was low positive reaction in epithelial lining renal tubules (Figure 1E), in summer there was very high positive reaction summer moderate reaction of large lymphocytes (Figure 4C) while at autumn low positive reaction was found in MMC.

Posterior kidney, at winter there was a high positive reaction of MMC and a moderate positive reaction in epithelial cells lining some renal tubules (Figure 4D), at spring there was low reaction in few renal tubules (Figure 4E), at summer there was a high positive reaction in capsule and macrophages and a moderate positive reaction in some renal tubules (Figure 4F) while at autumn a shallow positive reaction was observed in renal tubules. From previously obtained results we can conclude that the positive reaction for both PPAR $\alpha$  and PPAR $\gamma$  reached its highest in the head kidney during winter while in the posterior kidney during the summer season. These results confirmed the vital role of both PPAR $\alpha$  and PPAR $\gamma$  in immune function especially during winter which considered as the most seasons in which fishes became more susceptible to infection by pathogens as cold weather act as a stress factor in fish (Press and Evensen 1999). As well as they have an essential role in controlling water resorption through renal tubules especially during summer seasons in which water and solute resorption increased, so we can conclude that both types of PPARs play an essential role in energy supply for immune function, water, and solutes resorption in catfish.

In zebrafish, PPAR $\alpha$  was high in tissues that perform their functions through catabolism of large amounts of lipids, for example, liver, kidney, and pancreas while PPAR $\gamma$  was weak in expression and was found only in intestine, pancreas, and gonads (Ibabe et al. 2002). In contrast, (Zheng et al. 2015) mentioned that the PPAR $\alpha$  expression was low in the kidney of adult yellow catfish. (Guan 2002) found that PPAR-alpha is expressed mainly in proximal renal tubules, PPAR $\gamma$  is mainly found in medullary collecting duct with low expression in renal glomeruli.

PPAR $\alpha$  was higher in expression than PPAR $\gamma$  in the posterior kidney of catfish, and both of them showed expression in epithelial lining renal tubules and melanomacrophage centers (MMC) which indicated the essential role of both PPAR $\alpha$  and PPAR $\gamma$  in resorption of water and solutes in renal tubules and providing the energy needed by MMC for the

in connective tissue capsule and epithelial lining renal tubules also the positive reaction was high in MMC, endothelial cells lining blood vessels and red blood cells (Figure 3F,G) and in autumn there was moderate positive reaction in epithelial lining renal tubules and high positive reaction in MMC, large lymphocytes and red blood cells (Figure 3H). The peroxisome proliferator receptors gamma (PPAR $\gamma$ ) also showed a positive reaction in the same sites as PPAR $\alpha$  and affected by season (Figure 4). Head kidney, at winter a high positive reaction of MMC and a moderate reaction of large lymphocytes and red blood cells, was observed (Figure 4A), at spring moderate reaction of MMC (Figure 4B), at immune mechanism. The PPAR $\alpha$  (Ibabe et al. 2004; Ibabe et al. 2005; Raingard et al. 2009) and PPAR $\gamma$  (KissTóth and Rószter 2008) have essential roles in the function of normal kidney via their roles in controlling mitochondrial and peroxisomal  $\beta$ -oxidation.

Seasonal changes of peroxisomes were previously reported in the liver of mullets, and the summer was found the season which has the highest level of peroxisomes expression (Orbea et al. 1999). The expression of PPARs gene differs throughout the year as the PPAR $\alpha$  expression was high in females only in May and was low in September than other months. PPAR $\beta$  expression was high in December in males only. PPAR $\gamma$  was more expressed in February in both males and females (Batistapinto et al. 2009).

The IFN $\gamma$  showed a positive reaction in both the head kidney and posterior kidney of African catfish, and it was observed that this positive reaction differed according to the season. In the head kidney, the positive reaction was found in the lymphocytes and melanomacrophage centers, this positive reaction was high at the winter, moderate at spring while it was low at the summer season (Figure 5) and very high at the autumn season. In the posterior kidney, the positive reaction was found in melanomacrophage centers and distal convoluted tubules. The IFN $\gamma$  showed the highest positive reaction in the posterior kidney during the autumn season and followed by winter. However, at spring the positive reaction was lower than winter and autumn but still high. Meanwhile, at summer low positive reaction was observed all over the kidney (Figure 6).

In the head kidney, during the winter season, the positive reaction was high and found in the connective tissue capsule and in the cytoplasm of some large T- lymphocytes which located under the capsule (Figure 5A). Also, the melanomacrophage centers showed a moderate reaction (Figure 5B). In the summer season, some large T- lymphocytes showed a high positive reaction in their cytoplasm (Figure 5C). However, the melanomacrophage centers showed a shallow positive reaction (Figure 5D). The positive reaction toward IFN $\gamma$  reach the highest level in the autumn season, it was very high in connective tissue capsule, the cytoplasm of T-lymphocytes and endothelial cells lining blood

vessels (Figure 5E), but was low in melanomacrophage centers (Figure 5F). So we can conclude that in the head kidney the T- lymphocytes and melanomacrophage centers showed high expression to IFN $\gamma$  which indicated that the IFN $\gamma$  acts its role in the immune response by stimulation of the macrophages and T- lymphocytes. This result is in agreement with (Zou et al., 2005; Chen et al. 2010; Yang et al. 2013; Pijanowski et al. 2015) as they reported that IFN $\gamma$  activates macrophages and neutrophils to enhance their antimicrobial activity and enhance antigen presentation and promoting the response of T-helper cell. Also, the macrophages can secrete anti-inflammatory cytokines in response to inflammation of the kidney (Ricardo et al. 2008).

In lower vertebrates, the structural and functional observation of IFN $\gamma$  indicated the presence of an innate, natural killer and adaptive immune responses (Savan et al. 2009). The IFN $\gamma$  expression in channel catfish indicated its presence in head kidney, thymus, spleen, intestine, and muscles while in zebrafish was found in the gills and intestine (Milevmilovanovic et al. 2006).

The IFN $\gamma$  expression in zebrafish was proved to be independent of the presence of the microorganism (Sieger et al. 2009). (Chang et al., 2013) Proved that IFN transcripts could be spliced intracellularly and produce IFNs which bind to intracellular IFN receptors. So enable infected cells to defense against viral pathogens.

In the posterior kidney, during the winter season the positive reaction was very high in the distal convoluted tubules (DCT) and melanomacrophage centers (MMC), while was moderate in proximal convoluted tubules (Figure 6A,B). In the spring season, a high positive reaction found in DCT and MMC, but a shallow positive reaction was found in other parts (Figure 6C, D).

During the summer season, a low positive reaction was found in DCT and MMC (Figure 6 E, F).

At autumn season resemble that in winter with some endothelial cells lining blood vessels and some T- lymphocytes showed a positive reaction (Figure 6 G, H).

In the posterior kidney the positive reaction to IFN $\gamma$  was found in the cytoplasm of the epithelium lining distal convoluted tubules, melanomacrophage centers, endothelial cells of some blood vessels and some cells inside these vessels, which confirm that IFN $\gamma$  secretion effects on the function of renal tubules in catfish. It was found that IFN $\gamma$  receptor plays a role in the regulation of renal major histocompatibility complex (MHC) in the renal tubules, arterial endothelium, and glomeruli, in response to inflammation and the basal state (Takei et al. 2000).

It was found that inflammatory cytokines including interferon-gamma are produced from Th1 (helper T- lymphocyte) cells under the effect of interleukin- 18 (IL18) (Okamura et al. 1998).

IL18 and IL18r are highly expressed in T cells,

macrophages, endothelial cells and smooth muscle cells (Mallat et al. 2001). IL18 act on Na-Cl co-transporter protein which expressed in distal convoluted tubules of the kidney for reuptake of NaCl and in turn controle inflammatory molecule expression, which confirmed close relationship between IFN $\gamma$  secretion and NaCl cotransporter protein expression in epithelial lining distal convoluted tubules of kidney (Rozansky et al. 2009).

#### 4. Conclusion

We can entirely conclude that IFN $\gamma$  secretion increased under stress condition induced by cold Weather. cold make fish more susceptible to invasion of pathogens and in turn IFN $\gamma$  activates T- lymphocytes and melanomacrophage centers to inactivate some viral and bacterial pathogens. As well as, effects on the functions of the renal tubules especially distal convoluted tubules by activation of NaCl cotransporter. This result confirms that IFN $\gamma$  mechanism of action in response to inflammation in fish resembles that previously described by other authors in mammals. Moreover, there is a reverse relationship was detected between PPARs and IFN $\gamma$  in both anterior and posterior kidneys of catfish.

Future work should be directed to investigate the diverse effect of high fat diet for fish at winter season.

#### Acknowledgment

This work was supported by the Fundamental Research Funds for the Central Universities of China (Project Number: 2662015PY033).

#### Conflict Of Interest

There is no conflict of interest.

#### Note

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