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Cytochrome P450 1A gene expression in response to therapies for the bacterial infection in fish.

Ahmed M. Hal [1] and Manal I. El-Barbary [2,*]

[1] Genetics and Genetic Engineering Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt
[2] Fish Diseases Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt

*** Correspondence Auther: manal278@yahoo.com**

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ABSTRACT

Cytochrome P450 1A (CYP1A), a cytochrome P450 gene, participates in the metabolism of xenobiotics. In this research, levels of CYP1A expression were evaluated in treated and untreated bacterial infections in *Oreochromis niloticus* organs and histopathological changes in splenic tissues. Twelve groups of *O. niloticus* fish: control (T_{1-4}), fish infected with *Aeromonas hydrophila* (T_{5-8}), and fish infected with *Pseudomonas fluorescens* (T_{9-12}) groups) were treated with *Nigella sativa* oil and ciprofloxacin. In the uninfected group, treatment doses did not affect CYP1A expression levels. In infected fish, the level of CYP1A expression significantly increased without treatment while relative CYP1A expression was significantly downregulated when treated with *N. sativa* oil and ciprofloxacin additives. The combined treatments had a synergetic effect on the downregulation of CYP1A expression in the liver and gill. The main histological alterations in the spleen of infected fish were severe hyperplasia of melanomacrophage centers (MMCs), which was higher in the *A. hydrophila* infected fish than in the *P. fluorescens* infected fish. The results clarify the mechanisms of CYP1A gene expression and MMCs against pathogens and their consequences.

INTRODUCTION

Cytochrome P450 genes (CYPs) produce closely related proteins to metabolize multiple endogenous compounds and xenobiotics. Cytochrome P450 activity takes place in the liver and other organs associated with inflammation and infection **(Bezirtzoglou, 2012; Stavropoulou *et al*., 2018)**. CYP450 alterations also affect cellular immunity in response to infection **(Morgan, 2009).** One of the CYPs, the CYP1A gene involved in carcinogen metabolism **(Androutsopoulos *et al*., 2009; Lu *et al*., 2020),** is a valuable indicator of the level of contamination **(Stegeman *et al*., 2001; Miller *et al*., 2004; El Barbary, 2018)**, a stressed condition **(Sharifian *et al*., 2020)**, and bacterial infections in *O. niloticus* organs **(Hal and El-Barbary, 2020).**

Most fish diseases with notable morbidities, mortalities, and subsequent severe economic losses are bacterial infectious diseases **(Olsson *et al*., 1998)**. Infections of *A. hydrophila* and *P. fluorescens* are known to be the primary cause of bacterial hemorrhagic septicemia in fish and are widely distributed in aquaculture, especially in a culture system **(El-Barbary and Hal, 2016).** Multiple procedures have been employed to combat these diseases including sanitary prophylaxis, disinfection, chemotherapy, and antibiotics such as ciprofloxacin which inhibit the action of bacterial topoisomerases, resulting in the rapid death of bacteria **(Romero *et al*., 2012).** In addition, the immunostimulants of plant sources have the ability to augment fish immunity in response to infectious diseases by enhancing specific and non-specific immunological defenses **(Harikrishnan *et al.*, 2011).** Several medicinal herbs have a stimulant and booster effect on the immunity of fishes, such as the extracts of *Nigella sativa* **(Awad *et al*., 2013)**. These exhibit useful antimicrobial activity against multi-drug resistant bacteria such as *Pseudomonas* and *Staphylococcus* resistant strains **(Mashhadian and Rakhshandeh, 2005; Morsi, 2000).**

Medicinal plants with an antimicrobial effect have different active components that can possibly alter the activity of cytochrome P450 enzymes **(Abd El-Aty *et al*., 2008)**. In the case of carbon tetrachloride exposure in rats, *N. sativa* oil offers biological protection through the suppression of CYPs **(Ibrahim *et al*., 2008).** The extracts of *N. sativa* interfere with CYP1A in vitro through inhibition of ethoxyresorufin O-de-ethylation reactions in rats and dogs **(Liu *et al*., 2013).** Moreover, an association has been observed between the density of MMCs, which is considered an immune function indicator, and CYP450 expression **(Passantino *et al*., 2014; van der Weiden *et al*., 1994)**. Increased density of MMCs has been found in fish whose livers have marked CYP1A expression **(Basilone *et al*., 2018). Tian *et al*. (2020)** identified CYP1A1 as an important regulator of macrophage phagocytic activity in bacterial infection. Although the role of cytochrome P450s in infectious and inflammatory states has been widely studied, the behavior and mechanism of CYP1A contributions to immunological responses still needs to be understood and requires further investigation.

This study therefore assesses the levels of CYP1A expression in *O. niloticus* organs and a histopathological examination of splenic tissue damage after bacterial infection with *N. sativa* oil and/or ciprofloxacin treatment-based diets. The potential changes in the levels of CYP1A expression in the treatment of fish bacterial infection may illuminate the role of CYP1A in the immunological response against fish pathogens.

MATERIALS AND METHODS

Experimental design

Experimental healthy *O. niloticus* fish were obtained from El-Serw fish farm, National Institute of Oceanography and Fisheries, and uniformly assigned to 12 groups (T_1-T_{12}, Table 1). Two duplicate aquaria were dedicated to each group to produce

biological replicates acclimated to aquaria that were fed a control diet for two weeks. Each aquarium (40 liters) contained eight fish (50 ± 2 g) and was supplied with de-chloride freshwater with continuous aeration. The bacterial load injected in a single dose in each fish contained 1×10^5 CFU resuspended in 100 µl sterile phosphate-buffered saline (PBS) for *A. hydrophila*, acc. no. LC208789 **(El-Barbary, 2017)** and *P. fluorescens*, acc. no. LC208785 **(El-Barbary & Hal, 2017).** Fish were anesthetized in clove oil solution before the operation **(Hamackova et al., 2006)**. Four groups (T_1-T_4) were control groups that were injected intraperitoneally (IP) with the same volume of 100 µl PBS while the other eight groups were injected IP with *A. hydrophila* (T_5-T_8) and *P. fluorescens* (T_9-T_{12}). Fish were fed on four tested diets comprising 30% protein twice daily at a feeding rate of 3% of body weight. The tested diets were: (1) a basal diet (BD) without supplemented additives for groups T_1, T_5, and T_9; (2) BD supplemented with *Nigella sativa* oil 7% for groups T_2, T_6, and T_{10}; (3) BD supplemented with ciprofloxacin 200 mg/kg for groups T_3, T_7, and T_{11}; and (4) BD supplemented with a combination of *N. sativa* oil and ciprofloxacin at the same dose for groups T_4, T_8, and T_{12}. The experiment was carried out for 14 days.

Table 1. Experimental design.

Group	Injection	Diet
T_1	Saline	Basal Diet
T_2	Saline	Basal Diet + *Nigella sativa* oil 70 ml/kg
T_3	Saline	Basal Diet + ciprofloxacin 200 mg/kg
T_4	Saline	Basal Diet + ciprofloxacin with *Nigella sativa* oil
T_5	*A. hydrophila*	Basal Diet
T_6	*A. hydrophila*	Basal Diet + *Nigella sativa* oil
T_7	*A. hydrophila*	Basal Diet + ciprofloxacin
T_8	*A. hydrophila*	Basal Diet + ciprofloxacin with *Nigella sativa* oil
T_9	*P. fluorescens*	Basal Diet
T_{10}	*P. fluorescens*	Basal Diet + *Nigella sativa* oil
T_{11}	*P. fluorescens*	Basal Diet + ciprofloxacin
T_{12}	*P. fluorescens*	Basal Diet + ciprofloxacin with *Nigella sativa* oil

Groups (T_1-T_4) Non-infected fish; Groups (T_5- T_8) and (T_9-T_{12}) were infected by *A. hydrophila* and *P. fluorescens*, respectively.

Histological examination

Three fish from each group were collected 14 days after the bacterial challenge; three spleens from each group were then sampled. These samples were fixed in 10% formalin, and the histological examination was performed in accordance with **Roberts (2012).** Using hematoxylin-eosin (HE), samples were stained and photographed using an ICC50 HD camera and a Leica LAS EZ microscope.

CYP1A gene expression analysis

The total RNA of *O. niloticus* organs (liver, kidney, gill, muscle, brain, and pituitary) was extracted using a total RNA purification kit (Jena Bioscience). Complementary DNA was synthesized using M-MuLV Reverse transcriptase (SibEnzyme Ltd.) to build the first strand of cDNA with an oligo-$dT_{12–18}$ primer (Bio Basic Inc.) in accordance with the manufacturer's instructions. To analyze the relative expression of CYP1A, the primer for the CYP1A gene (**F**-GCAAATGGCTGCTGCTTGTCA and **R**-GTGTATCAAGGGTTCATGCCCT) and β-actin gene as an internal control (**F**-TCAGGGTGTGATGGTGGGTATG and **R**-CTCAGCTCGTTGTAGAAGGTGT) were used for real-time PCR amplification. Real-time PCR reactions took place in a total volume of 20 µl containing 4 µl HOT FIREPol EvaGreen qPCR Mix Plus (5X), 2 µl cDNA (200 ng/µl), 0.5 µl each for forward/reverse primers, and 13 µl H_2O PCR grade. Real-time PCR was performed at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, and annealing at 62°C for 15 s and 72°C for 12 s. Melting curves were obtained in a Stratagene MX3005P (ABI, CA, USA) from 65°C to 95°C. The relative CYP1A expression was calculated using the $2^{-\Delta\Delta CT}$ method **(Livak and Schmittgen, 2001)** normalized to β-actin. The expression of CYP1A was represented as the average ± standard error (in triplicate) of the sample replicates.

Statistical analysis:

Using IBM SPSS (Version 19), multiple comparisons of gene expression in fish organs were performed with one-way ANOVA followed by Duncan's test.

RESULTS

1. Clinical examination of infected fish

Several *A. hydrophila* and *P. fluorescens* infected *O. niloticus* samples exhibited signs of septicemia such as multiple skin ulcers, scale detachment, hemorrhage, cloudiness of eyes, diffuse abdominal distention, and anal inflammatory changes. Samples of *P. fluorescens* infected fish were characterized by congestion and enlargement of internal organs such as the kidney, spleen, and liver**.**

2. Histopathological examination

In Plates 1 & 2, no histopathological changes were detected in control group spleens (T_1) (Plate 1a,b) or nearly detected in groups fed on supplemented diets that presented a normal formation of red and white pulps (T_2-T_4). In *A. hydrophila* infected group T_5, the splenic tissue exhibited intense hyperplasia of melanomacrophage centers, infiltration of fibroblasts and necrosis, (Plate 1c). Splenic tissues in infected group such as T_9 displayed necrosis (Plate 2a), hemolytic areas in between red pulps with vacuoles and hemosiderin deposits (Plate 2b). The spleen of *A. hydrophila* infected fish groups fed on tested supplemented additives, *N. sativa* oil, ciprofloxacin, and a combination of them (T_6, T_7, & T_8, respectively) exhibited hemorrhage between splenic pulps and inflammatory cells, accumulations of MMCs, and necrosis (T_6, Plate 1d). The spleen in

group T_7 displayed severe vacuolation and degeneration of splenic cells, severe hemolysis between pulps, and accumulations of hemosiderin (Plate 1e). In addition to severe accumulations of MMCs and infiltration of fibroblasts found in group T_8 (Plate 1f), splenic tissues in *P. fluorescens* infected group T_9 exhibited necrosis (Plate 2a) hemolytic areas between red pulps with vacuoles, hemosiderin deposits, and disrupted blood vessels surrounded by fibroblast infiltrates (Plate 2b). These histological alterations in the spleen of *P. fluorescens* infected fish were similar to those observed in treated groups T_{10}-T_{12} which fed on the *N. sativa* oil, ciprofloxacin, and a combination of the two, respectively; the spleen in T_{10} exhibited severe accumulation of MMCs and inflammatory cells (Plate 2c) and severe vacuolation with the deposition of hemosiderin (Plate 2d). The spleen in group T_{11} exhibited necrosis and severe accumulation of MMCs (Plate 2e) while the spleen in T_{12} displayed infiltration of erythrocytes and inflammatory cells, hemolysis between splenic pulps, and deposition of hemosiderin (Plate 2f). In general, the histological alterations reduced due to the use of supplemented additives.

3. CYP1A expression of O. niloticus organs

In uninfected fish group $T_{1\text{-}4}$, treatment with therapeutic additives did not cause any significant alteration in CYP1A expression levels compared to the control (T_1) in the organs studied. In infected untreated fish T_5 & T_9, the relative expression of CYP1A significantly increased in all organs studied except the pituitary, where the expression was significantly downregulated (T_5 & T_9), while the expression in the brain was not affected in the *P. fluorescens* infected fish (T_9) compared to the control (T_1). After *A. hydrophila* infection ($T_{5\text{-}8}$), *N. sativa* oil led to a significant downregulation of CYP1A gene expression in the liver from 13.5 to 8.4 fold, the kidney from 8 to 4.6 fold, the gill from 6.2 to 4.3 fold, the muscle from 1.5 to 0.8 fold, and the brain from 1.7 to 1.4 fold, and increased CYP1A expression in the pituitary from 0.8 to 1.4 fold ($p < 0.05$; Fig. 1). Additionally, ciprofloxacin significantly decreased CYP1A expression levels after *A. hydrophila* infection in all studied organs of *O. niloticus*. After *P. fluorescens* infection ($T_{9\text{-}12}$), *N. sativa* oil decreased CYP1A expression levels in the liver from 18.6 to 5.3 fold, the kidney from 7.4 to 5.8 fold, the gill from 4.7 to 2.3 fold, and the muscle from 1.5 to 0.8 fold, and resulted in a non-significant increase in the level of CYP1A expression in the brain (T_{10}). The relative expression of CYP1A decreased with ciprofloxacin treatment from 18.6 to 6.1 fold in the liver, 7.4 to 6.3 fold in the kidney, 4.7 to 3.2 fold in the gill, and 1.5 to 0.6 fold in the muscle. The combined therapeutic additives (*N. sativa* oil and ciprofloxacin) significantly decreased levels of CYP1A expression in the liver, kidney, gill and muscle (T_8 &T_{12}) compared to the infected untreated fish (T_5 &T_9), but significantly increased in the pituitary in both infection types (Fig. 1).

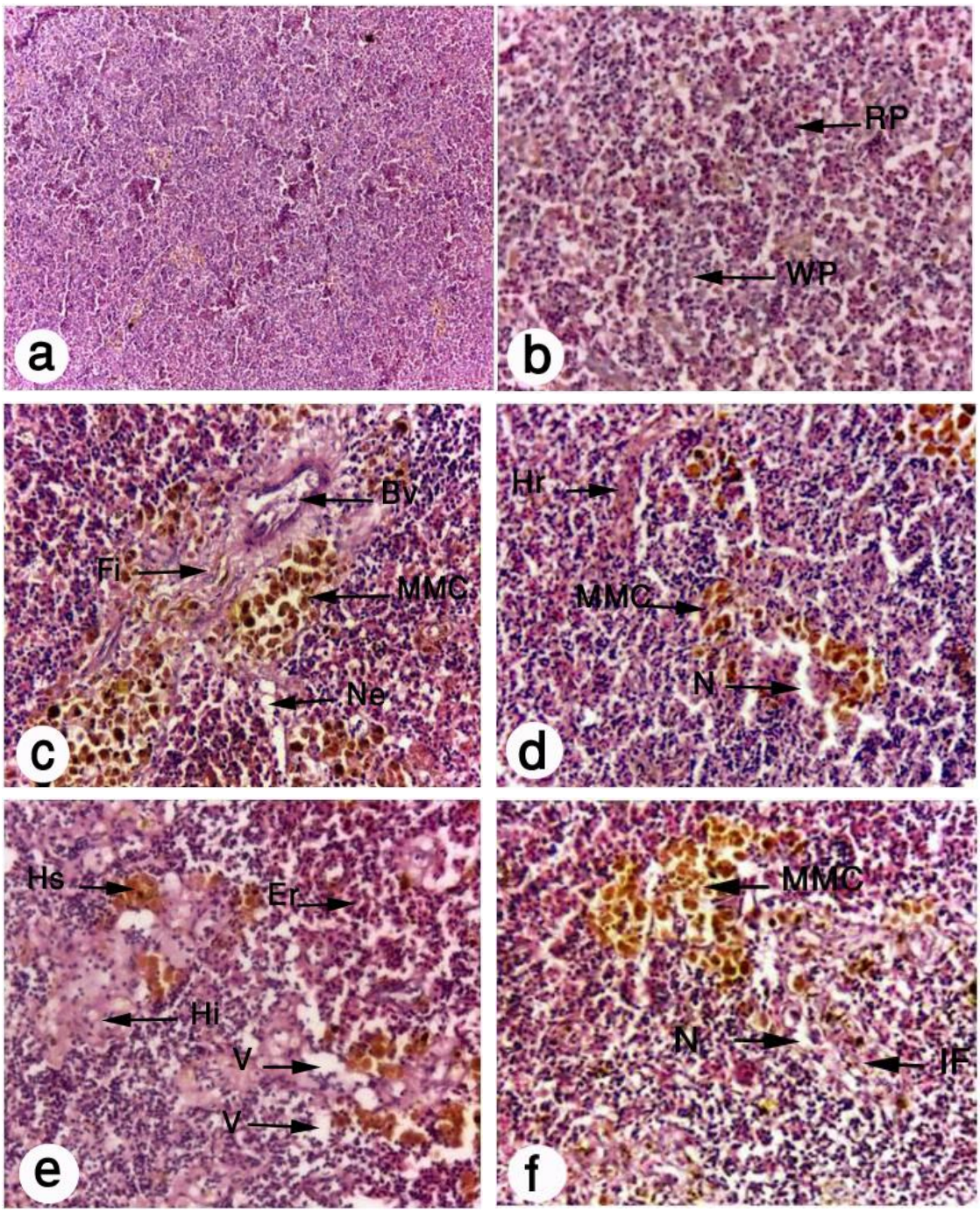

Plate 1 Histopathological changes in the spleen of control group and *A. hydrophila* infected *O niloticus* (T_1-T_8) stained with H&E.; (a, b) T_1 showed normal structure of red and white pulps in spleen (x100, 200). (c) T_5 showed infiltration of fibroblasts between splenic pulps, severe accumulation of MMC around damage blood vessels and necrosis (x200). T_6 showed hemorrhage, inflammatory cells and accumulations of MMC with necrosis (d x200). T_7 exhibited vacuolation and degeneration of splenic cells, severe hemolytic between pulps and accumulations of hemosiderin (e x200). T_8 exhibited severe accumulations of MMC and infiltration of fibroblasts (f x200). WP= white pulp, RP= red pulp, MMC= melanomacrophage cells, Fi= fibroblasts, Bv= blood vessel, He= hemorrhage, N= necrosis, Hi= hemolysis, Hs= hemosiderin.

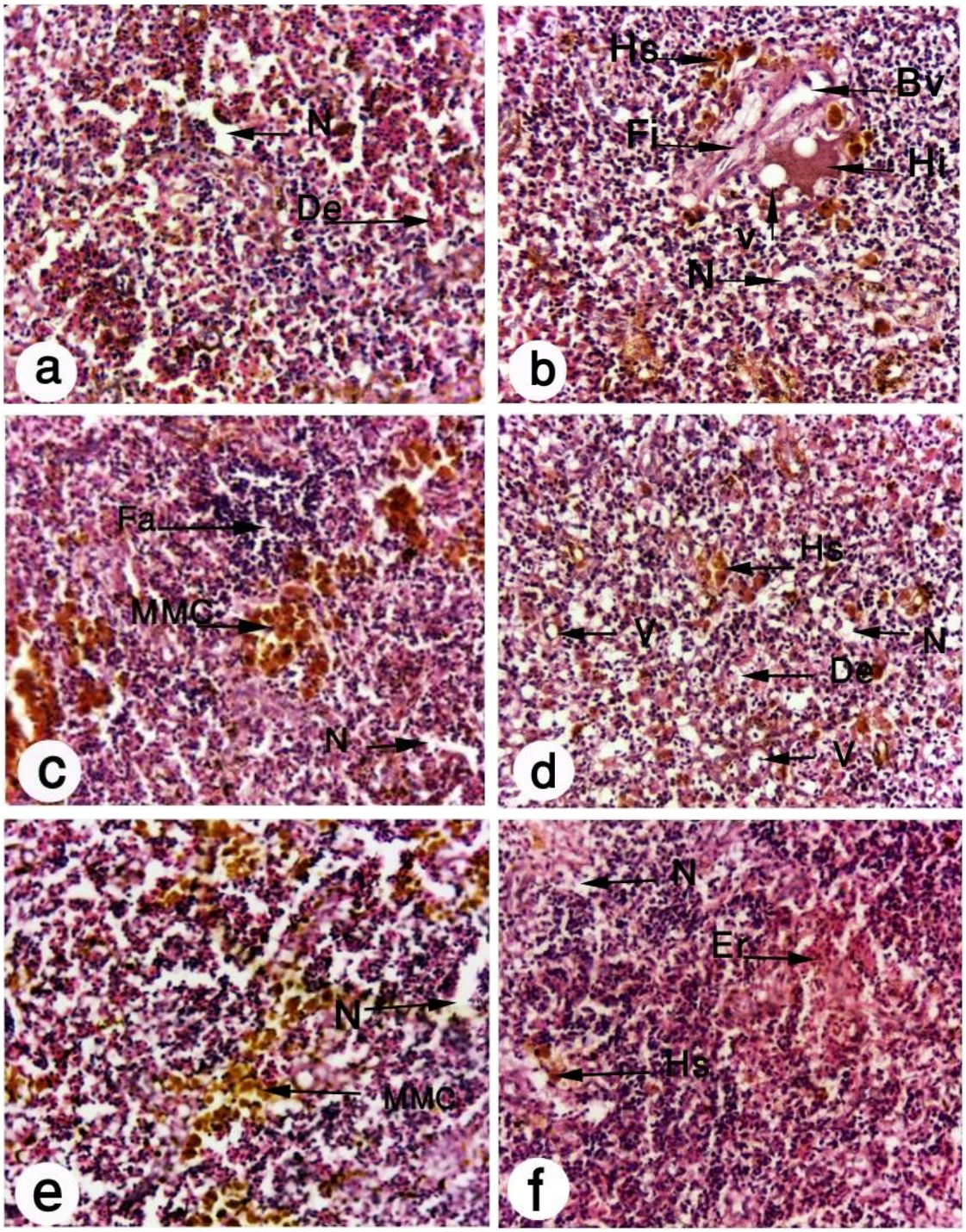

Plate 2 Histopathological changes in the spleen of *P. fluorscens* infected *O niloticus* (T_9-T_{12}) stained with H&E.; (a, b) T_9 showed necrosis (a x200) and infiltration of fibroblasts around damage blood vessels, hemolytic and vacuolation with deposition of hemosiderin (b x200). (c, d) T_{10} exhibited severe accumulation of (MMCs) and inflammatory cells (c x200) severe vacuolation with deposition of hemosiderin (dx200) T_{11} showed necrosis and severe accumulation of (MMCs) (e x200). T_{12} showed infiltration of inflammatory cells and erythrocytes, hemolytic between red pulps and deposition of hemosiderin (fx200). WP= white pulp, RP= red pulp, MMC= melanomacrophage cells, Fi= fibroblasts, Bv= blood vessel, He= hemorrhage, N= necrosis, Hi= hemolysis, Hs= hemosiderin. Er= erythrocytes.

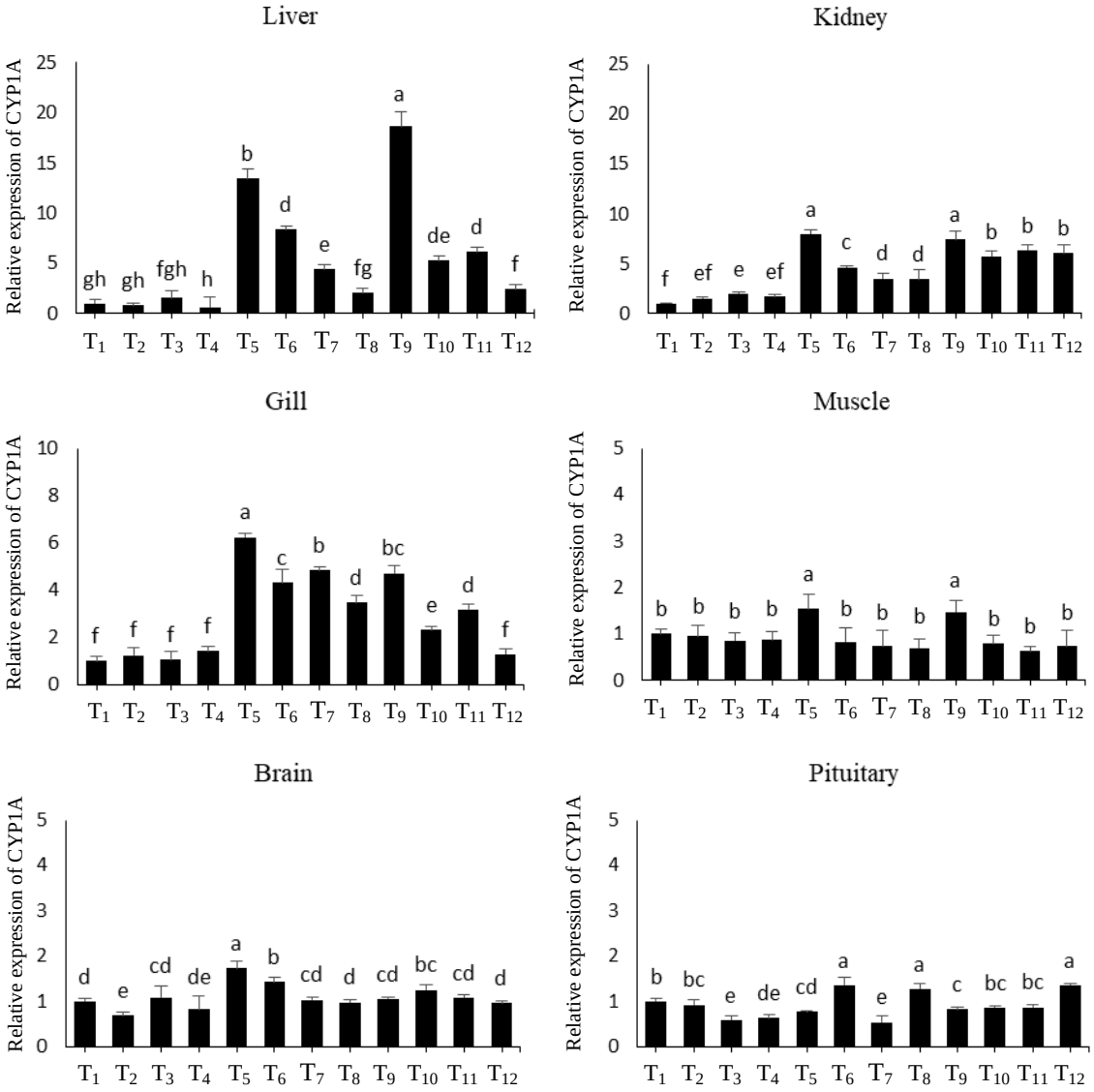

Figure 1. The relative expression of CYP1A of *O. niloticus* studied organs in untreated uninfected fish (T_1), treated uninfected fish (T_2-$_4$), with *N. sativa* oil (T_2, T_6 and T_{10}), ciprofloxacin (T_3, T_7 and T_{11}), and the combination of both (T_4, T_8 and T_{12}) after infection with *A. hydrophila* (T_5-$_8$) and *P. fluorescens* (T_9-$_{12}$). Above each column, different letters indicate a significant difference ($P < 0.05$).

DISCUSSION

The clinical symptoms in this experimental infection were cloudiness of eyes, scale detachment, ulcers on the skin, hemorrhage, inflammation of the anus, and abdominal distention in some *O. niloticus*. The *A. hydrophila* and *P. fluorescens* infected fish were characterized by congestion and enlargement of the liver, spleen, and kidney. Some of these signs are consistent with those observed by **El-Barbary (2010a, 2010b) and Yambot and Inglis (1994)** whose reported that *A. hydrophila* infection resulted in

acute mortality and obvious signs such as cloudiness in one or both eyes, which is associated with exophthalmia. Additionally, **Hardi and Pebrianto (2012)** reported that the *Aeromonas* infection caused the fish to bleed on the surface of infected organs such as the body, skin, and operculum, while the clinical signs of *Pseudomonas* sp. are mostly indicated by pale and watery internal organs, and gall bladder ruptures. In infected *O. niloticus*, each *Pseudomonas* species and *A. hydrophila* produce toxins and enzymes, such as protease, hemolysins, enterotoxins, cytotoxins, and others which are responsible for their pathogenicity and virulence **(Abou El-Geit *et al*., 2013; Eissa *et al*., 2010; El-Barbary, 2010a, 2017; El-Barbary and Hal, 2017).**

Histopathological examination is a well-established tool utilized to assess the structure and magnitude of tissue changes caused by biological and chemical insults and represents a fair biological marker **(Camargo and Martinez, 2007).** The spleen has two major components: a lymphopoietic portion (white pulp): a hematopoietic part (red pulp) that make an obvious immunological contribution in fish **(Fournier-Betz *et al*., 2000; Kurtović *et al*., 2008; Lin *et al*., 2005)**, where it is the only lymph-node in bony fish **(Roberts, 2012).**

In the spleen, notable hypertrophy and hyperplasia of MMCs occurs in response to bacterial infection **(Alagappan *et al*., 2009; Soto *et al*., 2009).** In the present study, the spleen of *O. niloticus* infected with *A. hydrophila* and *P. fluorescens* exhibited severe hyperplasia of MMCs, infiltration of fibroblasts, and necrosis. In Plates 1, 2, the spleen of infected *O. niloticus* exhibited deposition of MMCs and hemosiderin that was also reported by **Chang & Plumb (1996)** and **Miyazaki and Kaige (1986)** who observed diffuse hemosiderin deposition in the spleen of carps infected by *A. hydrophila.* MMCs may therefore contribute to humoral adaptive immunity as they are the evolutionary precursors of the mammalian germinal centers **(Steinel and Bolnick, 2017)** and appeared dark brown with areas patchy of hemopoietic portion depletion **(Mahmoud *et al*., 2018).** In addition, histological changes in the spleen identified severe hyperplasia of MMCs in both *A. hydrophila* and *P. fluorescens* infected fish (T_5 &T_9 respectively). These findings may be attributable to the production by *P. fluorescens* of extracellular proteases, which have a deleterious effect on endothelium and parenchyma that results in pathological hemorrhages and degenerations **(Miyazaki *et al*., 1984).** Furthermore, various extracellular toxins and enzymes linked to *A. hydrophila* and added to other environmental toxicants may be responsible for these lesions **(Garcia-Abiado *et al*., 2004; Gogal *et al*., 1999)**. Hemolysis in the infected groups (Plates 1e & 2f) may be due to damage to erythrocytes membranes **(Hibiya, 1982; Scott and Rogers, 1981; Zapata and Cooper, 1990)** where abnormal movement and aggregation of intra-splenic erythrocytes is an aspect of splenic congestion and a cause of hemolysis.

In general, the histological alterations were reduced when supplemented additives were used. *N. sativa* is reported to have antibacterial actions against a wide range of microorganisms **(Sokmen *et al*., 1999; Soliman *et al*., 2017)** as well as anti-in-

flammatory action **(Mutabagani & El-Mahdy, 1997)**, renal-protective **(El Daly, 1998),** hepatoprotective **(Daba & Abdel-Rahman, 1998)**, and immune-potentiating properties **(Swamy and Tan, 2000)**. Similarly, **Kaleeswaran *et al*. (2012)** observed that aggregations of splenic MMCs were more evident in experimental groups of *Catla catla* that fed on the medicinal plants *Cynodon dactylon* than in the control group, which explains the subsequent inhibition of *A. hydrophila* growth. **Mahmoud *et al*. (2014)** also reported increased MMCs activity in fish spleen treated with spirulina which appears to strongly enhance immunity. Additionally, *N. sativa* oil enhanced the immune response and protection of *A. testudineus* against *A. hydrophila* **(Khatun *et al*., 2015)** and its action as a disease controlling agent for *C. carpio* against *P. fluorescens* **(Khondoker *et al*., 2016).**

In this study, the increased density of MMCs went along with CYP1A overexpression. Our findings align with previous reports of an association between MMCs and CYP1A in stress conditions and apoptosis **(Corriero *et al*., 2013; Passantino *et al*., 2014; van der Weiden *et al*., 1994).**

Activities of cytochrome P450s were affected by infection and inflammation with an observed interplay with inflammatory mediators. Several studies have reported a relationship between pollutant-related genes and immunity defense **(Brown *et al*., 2006; Hal & El-Barbary, 2021a,2021b; Wang *et al*., 2009).** In this study, the relative expression of CYP1A increased in all organs of untreated infected fish except for the pituitary in both infection types and the brain in *P. fluorescens* infection. Although not all previous studies reported upregulation of CYP1 levels due to infection (e.g., **Neyrinck *et al*., 2009; Renton and Nicholson, 2000; Tindberg *et al*., 2004**), other studies have reported upregulation of CYP1 levels in response to infection and inflammation **(Hussain *et al*., 2014; Lee *et al*., 2015; Schiering *et al*., 2017; Tian *et al*., 2020).** Even in response to parasitic diseases, an increase in total CYP450 content has been detected and the CYP1A1 activity upregulated in rat liver, as observed by **Montero *et al*. (2003)**. **Divanovic *et al*. (2013)** reported that the CYP gene is involved in the metabolism of lipid mediators responsible for the initiation and resolution phase of inflammation and noted that CYP1 monooxygenase participates in their biosynthesis and inactivation and modulates their balance in the inflammatory process. Furthermore, there is an association among CYP1A over expression, down regulation of proinflammatory cytokines, and activation of the PPAR-γ signal pathway, which exerts anti-inflammatory action in pigs **(Fang *et al*., 2016).**

With *N. sativa* oil treatment, there was a decrease in the levels of CYP1A expression in organs. This aligns with the observations of **Ibrahim *et al*. (2008)** who reported that *N. sativa* oil offers biological protection with a suppression effect on CYPs in carbon tetrachloride exposure in rats. In addition, *N. sativa* inhibited CYP1A catalytic activity in the liver of canines and murines **(Liu *et al*., 2013)** and decreased relative CYP1A expression in the blood of tilapia after the infection **(Hal & El-Barbary, 2021**a).

In our study, ciprofloxacin decreased CYP1A expression levels after the bacterial infection. Similarly, expressed zebra fish CYP1A has been found to be inhibited by ciprofloxacin **(Smith *et al*., 2012),** which exhibited a selective suppression of CYP1A2 **(Granfors *et al*., 2004)**. Our results indicated that the therapeutic effect of additives (*N. sativa* oil and ciprofloxacin) led to down regulation of CYP1A expression with control of the infection, suggesting that the over expression of CYP may exhaust the immune defense and that down regulation has a protective effect in the bacterial infection. This was observed by **Schiering *et al*. (2017)** who reported that exaggerated over expression of CYP may result in undesirable inflammatory sequences with depletion of the aryl hydrocarbon receptor (AHR) tissue store. This hampers the AHR-dependent immune response which, in turn, increases mortality in *Citrobacter rodentium* infection due to functional depletion of AHR.

Alterations of P450 enzyme activities in the brain by infection or inflammation might therefore modify responses in the central nervous system (CNS). Fish CNS are observed to have a blood-brain barrier structurally similar to the mammalian form with the exception of the hypothalamohypophyseal area. This implies selective permission of systemic mediators and drug entrance to the brain **(Orellana-Paucar *et al*., 2013; Tenor *et al*., 2015)**, which has an effect on the CNS behavior of cytochrome levels in terms of stress and inflammatory status. In the current study, the alteration of the relative expression of CYP1A in the brain was slightly increased by infection and decreased by treatments in the *A. hydrophila* infection, while the expression did not significantly change in the *P. fluorescens* infection group. It has been reported that the levels of brain cytochrome P450 are low with difficult detection of various isoenzymes and nonselective LPS stimulation of several cytochrome isoenzymes **(Monshouwer *et al*., 2000).** In other studies, CYP1A was downregulated in response to lipopolysaccharide injection in the cerebroventricular compartment **(Renton & Nicholson, 2000)** and LPS injection in the brain affected hepatic cytochrome enzymatic activity **(Shimamoto *et al*., 1998).**

In our study, CYP1A expression in the pituitary exhibited different behavior, as it decreased in infection without treatment compared to CYP1A expression in other organs. In fish stress and infection, hypothalamic-pituitary-adrenal axis was observed to have an immune regulatory action through feedback mechanisms in a hypothalamic-pituitary axis in response to systemic cortisol, catecholamine, and cytokine levels **(Holland *et al*., 2002; Verburg-van Kemenade *et al*., 2017),** which may cause a negative feedback effect with decreased metabolic activity. Furthermore, fish exhibited a IL-1 receptor in the pituitary and hypothalamus, which is involved in the stress response **(Metz *et al*., 2006; Pijanowski *et al*., 2015).**

CONCLUSION

The histopathological examination of spleen tissue of *O. niloticus* infected by *A. hydrophila* and *P. fluorescens* showed that all lesions were observed in all infected

groups. The severe hyperplasia of MMCs, infiltration, and necrosis were higher in the *A. hydrophila* infected fish group than the *P. fluorescens* group. However, these histopathological alterations reduced with the use of *N. sativa* oil and/or ciprofloxacin. Relative CYP1A expression increased in all studied organs of untreated infected fish. The therapeutic additives (*N. sativa* oil and ciprofloxacin) led to downregulation of CYP1A expression after infection. However, CYP1A expression exhibits different behavior in the pituitary. These results could help illuminate the mechanisms of CYP1A gene expression and MMCs against the pathogens and their consequences.

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