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Pathological and Biochemical Studies on some Antimicrobials in *Clarias garipeinus* Fish Infected with *Aeromonas hydrophila*

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Abstract

The present study was carried out to investigate the efficacy of propolis and norfloxacin against *Aeromonas hydrophila* in Nile catfish (*Clarias garipeinus*). Fish were collected from a private fish farm in Sharkia Governorate and fed commercial fish diet. Fish were divided into six groups; Group 1: non-infected non-treated, Group 2: experimentally infected with *A. hydrophila* and non-treated, Group 3: normal fish administered propolis in feed by dose (10g/kg BW for 10 days), Group 4: infected fish treated with therapeutic dose of norfloxacin (10mg/kg BW for 10 days), Group 5: infected fish treated with propolis and Group 6: infected fish treated with therapeutic dose of norfloxacin and propolis (with the previous dose). The results indicated that propolis and norfloxacin were effective against *A. hydrophila*. The hematological parameters were improved in Groups 4, 5 and 6 when compared with Group 2. The second group showed a significant increase ($p<0.05$) in serum aspartate aminotransferase (AST), alanine aminotransferrase (ALT), urea, creatinine and malondialdhyde activity, while the mentioned parameters were improved decreased in Groups 5 and 6. Also, our results revealed a significant increase ($p<0.05$) in immunological parameters in Groups 3, 5 and 6. Moreover, this study also reported the pathological lesions in gills, liver, kidneys, heart, spleen and intestine of fish infected with *A. hydrophila* which became milder in treated fish especially with propolis and antibiotic. The present results suggest that the administration of propolis and norfloxacin were effective against *A. hydrophila* without hazard effects on hematological and biochemical parameters.

Keywords: Propolis, *Aeromonas hydrophila,* Catfish, Norfloxacin.

Introduction

Fish is considered the cheapest source of animal protein; therefore, most countries are paying a great attention to improve their inlet resources to satisfy their requirements of animal protein [1]. Propolis is a natural honeybees' product, which contain a variety of different chemical compounds as polyphenols (flavonoid aglycones, phenolic and their esters, phenolic aldehydes, alcohols and ketones), steroids, amino acids and inorganic compounds [2]. Propolis is aresinous material produced by worker bees from leaf bud and exudates of plants [3]. It has many different pharmacological activities as anti-inflammatory, antiviral, antioxidant, antifungal, antibiotic and immunostimulant effects [4]. In a recent study on *Oreochromis niloticus* (*O. niloticus*), propolis-ethanolic-extract enhanced the growth, immunity and resistance against *Aeromonas hydrophila* more than the crude propolis [5].

Quinolones are bactericidal broad-spectrum antibacterial agents that act especially against gram negative bacteria that inhibit bacterial growth by interfering with the DNA gyrase. They have low minimum inhibitory concentration (MIC) value for most susceptible fish pathogens and effective systemic distribution in fish when administered orally via medicated feed [6]. The antimicrobial spectrum of norfloxacin makes this drug attractive in veterinary therapy [7]. *A. hydrophila* infection is the scourge of fresh and warm water fish farming worldwide and is considered as a significant economic problem [8].

Biochemical, hematological and immunological parameters of fish are

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considered as an index of their health status. Fish are mostly used to predict the influence of the environmental pollutants due to owing to their higher biological sensitivity, which can be measured biochemically and hematologically under some stress cases [9]. The histopathological examination on Nile catfish *(Clarias garipeinus)* with *A. hydrophila* infection represented sever hepatic and renal lesions as degenerative necrotic changes, hemosiderosis, hemorrhages in liver and coagulative necrosis in kidney [10]. The objective of the present study was to investigate the influence of dietary supplementation with propolis and norfloxacin on hematological, biochemical and immunological parameters in African catfish *Clarias garipeinus* infected with Aeromonas *hydrophila*

Material and Methods

Sensitivity test and experimental design

Disc diffusion method was carried according to Bauer *et al*. [11]. The antibiotic discs were Gentamycin, 10 µg, Norfloxacin, 10 µg, Amoxicillin, 30 µg and Erthromycin, 15 µg. The technique was according to the standardized National Committee for Clinical Laboratory Standards [12]. Sixty Nile catfish *(Clarias garipeinus)* were obtained from a private fish farm in Sharkia Governorate of weight and length ranged between 55-70 gm, 23-30 cm, respectively. They were divided into six equal groups; Group 1 normal healthy fish non-infected non-treated (negative control), Group 2; fish inoculated intraperitoneally with 0.2 mL of 24 h broth cultures of *Aeromonas hydrophila* (2.5×10^8 Ml, obtained from Animal Health Institute Dokki, Cairo. Preserved on semisolid agar at refrigerator) and kept without medication (positive control), Group 3 normal healthy fish fed on diet supplemented with propolis (Propolis powder, Ethanolic extract 70%, plant protection research institute (PPRI)) at 10 g/kg diet for 10 days [13].

Groups 4, 5 and 6 were inoculated intraperitoneally with 0.2 mL of 24 h broth cultures of *A. hydrophila* (2.5×10^8 mL) and then fed on diet supplemented with norfloxacin (Atonor® each ml contains 300 mg of norfloxacin, ATCO Pharma, EGYPT) at 10 mg/kg diet for 10 days (Group 4) [6], propolis 10 g/kg diet for 10 days (Group 5) and simultaneously with a therapeutic dose of norfloxacin plus propolis (Group 6), respectively. They were kept in a well aerated glass aquarium to be acclimatized on dechlorinated tap water for two weeks. Each aquarium was supplied with air pump and water temperature was fixed at 27±2ºC, PH was 7-8.5. Fish were fed on commercial pelleted ration once daily at rate of 2% body weight.

Blood samples

Three blood samples were collected from each group from caudal vein under aseptic condition after 1 and 10 days post treatment. The first blood sample was collected on EDTA for hematological examination (1 mL). The second blood sample was collected in a sterile plastic tube containing heparin to be used for phagocytic activity investigation (2 mL), while the third blood sample was taken without anticoagulant in a clean and dry centrifuge tube (3 mL), left to clot at room temperature and centrifuged at 3000 rpm for 5 min. Serum was collected, labeled, placed in dry clean-capped tubes and frozen at -20^{o}C for biochemical analysis.

The hematological and biochemical study

The erythrocytic count, hemoglobin concentration, packed cell volume and total leucocytic count were carried out using automatic cell counter for veterinary use (Sysmex XT-2000iv). Ddifferential leucocytic counts were calculated according to Cole [14]. Test kits were used for estimating liver enzyme activity (serum alanine aminotransferase ALT and serum aspartate aminotransferase AST) [15], serum urea [16], serum creatinine [17], serum total protein [18] serum albumin [19] and L-Malondialdehyde (MDA) [20]. The serum globulin was calculated by subtracting albumin level from total protein level.

Phagocytic activity and index

To determine the phagocytic activity, the peripheral blood mononuclear cells (PBMC) were isolated [21]. Then added 0.25 mL of adjusted viable leukocytes suspension to 0.25 mL heat inactivated *Candida albicans* (*C. albicans*) in serial plastic tubes. The tubes were incubated at 37°C for 30 minutes in a

humidified Co_2 incubator. Subsequently, the tubes were centrifugated at 2500 rpm for 5 minutes and the supernatant was removed with Pasteur pipette leaving a drop in which the sediment was re-suspended. Smears were prepared from the deposit, dried in air and stained with Leishman´s stain [22]. Under a light microscope using oil immersion lens, a total number of 100 phagocytic cells were counted randomly in about ten microscopic fields. The number of ingested yeast cells in each individual phagocytes were determined to calculate the phagocytic ratio in each of the tested group. The phagocytic ratio is considered as the percentage of phagocytic cells by microscope field, while the phagocytic index is the mean number of *C. albicans*, ingested by one phagocytic cell [22].

Histopathological investigation

The macroscopic and microscopic findings were recorded. The collected specimens from gills, liver, heart, kidneys, spleen and intestine were fixed in 10% formalin solution, Paraffin sections of 5-micron thickness were prepared and stained with hematoxalyin and eosin [23] and then examined microscopically.

Statistical analysis

The data obtained from this investigation were statistically analyzed by F-test [24] using MSTAT-C computer program.

Results and Discussion

In-Vitro sensitivity test of *A. hydrophila* strain against antibiotic using agar disc diffusion method showed that *A. hydrophila* was susceptible to norfloxacin with clear zone of inhibition (18 mm). The obtained result is similar to previously detected by El-Deen and Mohamed [25] who recorded that in vitro, *A. hydrophila* was sensitive to norfloxacin and enrofloxacin. Treatment with norfloxacin was effective and increased the survival of fish challenged with *A. hydrophila*. Antibiotics of the family quinolones (norfloxacin and enrofloxacin) and gentamicin proved to be the most efficacious on *A. hydrophila* isolates [26]. The experimentally infected fish with *A. hydrophila* in the current study was responded to propolis and norfloxacin treatment. The mortality rate reached 80% at the 9^{th} day post infection in infected non-treated group, while the medicated groups showed reduction in mortality rate (15-20%).

Administration of propolis or propolis with norfloxacin was effective against *Areomonas* infection in fish. Gram-positive and Gram-negative bacteria were sensitive to propolis but the Gram-negative was more sensitive [26]. Abd-El-Rhman [5] studied that propolis had antagonistic effect against Aeromonas infection in fish. Moreover, propolis had synergistic effects with antibiotics like chloramphenicol, neomycin and tetracycline [27]. The antibacterial activities of propolis extracts were related to phenolic contents [28].

Our results indicated that infected non-treated fish with *A. hydrophila* (Group 2) revealed a significant decrease in the erythrocytic count, Hb concentration and packed cell volume. On the other hand, there was a significant increase in the leucocytic count and lymphocyte at two experimental periods (1^{st} and 10^{th} day post treatment) (Table 1). The current results were in accordance with the results previously obtained by Ahmed [29] and Amer *et al*. [30] who found that *Clarias lazera* infected with *A. hydrophila* induced a significant decrease in the erythrocytic count, Hb concentration and packed cell volume. These changes are due to the *A. hydrophila* pathogenesis which reported to involve variety of biological activity extracellular products and enzymes including cytotoxins, hemolysis, proteases and enterotoxins which are believed to be associated with *A. hydrophila* virulence [31]. The elevation of total leucocytic count could be due to antigen stimulation by bacterial infection [14].

Table 1: The effect of propolis and norfloxacin (mean±SE) on erythrogram and leukogram of clinically healthy and infected *Clarias garpennius* with *Aeromonas hydrophila*.

	Groups	RBCs[1] (10^6×mm^3)	Hb[2] (g/dL)	PCV%[3]	WBCs[4] (10^3×mm^3)	Differential leucocytic count%				
						Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
1st day post treatment	1	2.38±0.03^{a}	11.26±0.20^{b}	27.05±0.16^{a}	23.26±0.22^{c}	60.03±0.66^{b}	30.92±0.64	5.72±0.07^{b}	1.89±0.02	1.44±0.04
	2	1.47±0.02^{c}	8.56±0.08^{e}	16.66±0.37^{b}	26.80±0.34^{a}	62.25±0.07^{a}	28.90±0.29	5.61±0.06^{b}	1.82±0.03	1.42±0.06
	3	2.37±0.02^{a}	11.66±0.08^{a}	26.78±0.15^{a}	25.87±0.40^{ab}	60.77±0.35^{b}	29.98±0.37	5.98±0.05^{a}	1.86±0.03	1.41±0.06
	4	2.04±0.03^{b}	10.03±0.05^{d}	26.44±0.18^{a}	25.96±0.43^{ab}	60.96±0.41^{ab}	30.15±0.33	5.64±0.06^{b}	1.89±0.01	1.36±0.04
	5	2.07±0.05^{b}	10.59±0.09^{c}	26.66±0.34^{a}	25.62±0.35^{b}	60.16±0.40^{b}	30.55±0.40	6.05±0.06^{a}	1.86±0.03	1.44±0.06
	6	2.06±0.06^{b}	10.34±0.12^{cd}	26.88±0.07^{a}	25.72±0.22^{b}	60.58±0.51^{b}	30.26±0.50	5.92±0.04^{a}	1.87±0.01	1.37±0.04
10th days post treatment	1	2.39±0.04^{a}	11.87±0.06^{a}	26.62±0.18^{ab}	23.72±0.32^{c}	60.92±0.64^{ab}	30.02±0.67	5.90±0.05^{c}	1.93±0.03	1.23±0.03
	2	1.80±0.07^{c}	8.69±0.09^{e}	16.58±0.29^{d}	26.60±0.29^{a}	62.01±0.26^{a}	29.13±0.31	5.69±0.07^{c}	1.91±0.03	1.26±0.03
	3	2.33±0.05^{a}	11.71±0.03^{a}	27.78±0.52^{a}	26.72±0.23^{a}	60.86±0.41^{ab}	29.84±0.35	6.16±0.07^{a}	1.90±0.02	1.24±0.03
	4	2.18±0.05^{b}	10.64±0.10^{d}	23.46±0.53^{c}	26.16±0.27^{a}	60.84±0.38^{bc}	30.05±0.45	5.95±0.07^{bc}	1.92±0.02	1.24±0.03
	5	2.30±0.12^{a}	11.32±0.16^{b}	26.56±0.19^{b}	24.26±0.32^{bc}	60.40±0.34^{bc}	30.18±0.39	6.14±0.06^{b}	1.95±0.02	1.33±0.03
	6	2.20±0.07^{b}	11.03±0.09^{c}	26.90±0.73^{ab}	24.80±0.24^{b}	59.50±0.22^{c}	31.20±0.18	6.08±0.08^{bc}	1.93±0.02	1.29±0.04

[1]RBCs: Red blood corpuscle, [2]Hb: Haemoglobin, [3] PCV%: Packed cell volume, [4]WBCs: White blood corpuscle
Means with different letters at the same column (1st and 10th days post treatment separately) were significant P<0.05.

Table 2: The effect of propolis and norfloxacin (mean±SE) on some biochemical parameters, phagocytic% and phagocytic index of clinically healthy and infected *Clarias garpennius* with *Aeromonas hydrophila.*

Group	1st day post treatment						10th day post treatment					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
AST[1] (U/L)	14.60±0.67^{d}	27.20±0.80^{a}	14.80±0.58^{d}	20.60±1.02^{b}	17.80±1.39^{c}	15.80±0.86^{cd}	15.2±0.86^{b}	27.8±0.86^{a}	14.6±1.02^{b}	16.8±0.86^{b}	16±0.09^{b}	15±0.63^{b}
ALT[2] (U/L)	17.40±0.92^{c}	29.60±0.50^{a}	17.35±1.02^{c}	24.60±0.50^{b}	20.40±1.63^{c}	20.42±1.80^{c}	16.60±0.92^{bc}	29.6±0.50^{a}	16±0.70^{c}	19.40±1.56^{b}	17.40±0.92^{bc}	19.20±0.86^{b}
Urea (mg/dL)	12.00±0.70^{c}	18.60±1.02^{a}	12.00±0.71^{c}	16.20±1.06^{ab}	13.20±1.28^{bc}	15.80±0.86^{ab}	11.40±0.67^{c}	19.20±1.01^{a}	11.80±0.86^{c}	15.20±0.66^{b}	12.60±1.24^{bc}	13.80±1.06^{bc}
Creatinine (mg/dL)	0.22±0.03^{b}	0.37±0.01^{a}	0.21±0.04^{b}	0.24±0.03^{b}	0.22±0.04^{b}	0.22±0.04^{b}	0.22±0.03^{b}	0.38±0.008^{a}	0.20±0.04^{b}	0.23±0.03^{b}	0.22±0.03^{b}	0.21±0.04^{b}
MDA[3] (mg%)	6.80±0.86^{b}	16.60±1.43^{a}	6.16±0.30^{b}	3.56±0.44^{b}	4.00±0.80^{b}	3.67±0.20^{b}	6.83±0.57^{b}	16.8±1.65^{a}	7.26±0.56^{b}	7.85±1.13^{b}	8.10±0.67^{b}	7.20±1.15^{b}
Total protein(g/dL)	4.04±0.18a	2.06±0.16c	4.1±0.17^{a}	3.08±0.24^{b}	3.54±0.18^{ab}	3.38±0.23^{b}	4.12±0.18^{a}	2.46±0.19^{b}	4.20±0.12^{a}	3.56±0.44a	4.00±0.80a	3.67±0.20a
Albumin (g/dL)	1.64±0.10	1.31±0.23	1.76±0.12	1.80±0.19	1.82±0.12	1.64±0.12	1.72±0.08	1.38±0.20	1.68±0.13	1.68±0.15	1.64±0.13	1.50±0.18
Globulin (g/dL)	2.40±0.15^{a}	0.74±0.13^{c}	2.34±0.14^{a}	1.28±0.30^{bc}	1.72±0.23^{ab}	1.73±0.28^{ab}	2.34±0.17^{a}	1.08±0.13^{b}	2.52±0.18^{a}	1.88±0.39^{a}	2.36±0.16a	2.22±0.09a
Phagocytic ratio	73.62±0.20^{d}	71.80±0.24e	74.26±0.15c	75.62±0.20^{b}	77.26±0.22a	75.60±0.17^{b}	75.42±0.25^{c}	69.30±0.37^{d}	78.26±0.42^{b}	74.28±0.53^{c}	79.68±0.19^{a}	78.68±0.19^{ab}
Phagocytic index	2.13±0.04^{d}	2.02±0.03d	2.13±0.04d	2.41±0.02^{bc}	2.52±0.01a	2.47±0.01^{ab}	2.29±0.03^{d}	2.07±0.03^{e}	2.47±0.02^{c}	2.48±0.02^{c}	2.70±0.04^{a}	2.56±0.02^{b}

[1]AST: aspartate aminotransferase, [2]ALT: alanine aminotransferase, [3]MDA: Malondialdhyde, Means with different letters at the same row were significant P<0.05.

Fish experimentally infected and treated with norfloxacin (Group 4) showed a significant increase in RBCs count, Hb concentration and PCV% when compared with infected non-treated group, which were in agreement with Mohamed [10] who found that florfenicol treatment improved the adverse effects of *A. hydrophila* infection on hematological parameters of Nile catfish *(Clarias garipeinus)*. On contrary, infected fish treated with propolis (Group 5) and propolis with norfloxacin (Group 6) showed an improvement in most of hematological parameters when compared with infected non-treated group. This may be due to chemical structure of propolis that including polyphenols, steroids, amino acids, protein, vitamins (A, B1, B2, B3 and biotin), minerals (iron, zinc, copper and cobalt) and inorganic compounds [2]. Our results were in agreement with Yonar *et al*. [32] who investigated the effects of propolis on oxytetracycline (OTC)-induced oxidative stress and immunosuppression in fish. Oxytetracycline had suppressive effect on specific and nonspecific immune system parameters, such as leukocyte counts, oxidative radical production, total plasma protein and immunoglobulin levels and phagocytic activity. Treatment with propolis (50 $mg.kg^{-1}$ body weight, orally) reduced the OTC-induced oxidative stress by importantly changing the levels of biochemical parameters in tissues. Upon the implementation of propolis, the compressed immune system parameters were significantly increased in fish exposed to OTC. In addition, propolis has immunostimulant effect and improved digestive utilization of iron with increased erythrocytic count [33].

In the current work, administration of propolis alone to non-infected (Group 3) and infected groups (Group 5) or in a combination with norfloxacin (Group 6) induced a significant increase in total leucocytic and monocyte count when compared with non-infected non treated group (Group 1). These results parallel to that reported by others [34,35] who found that propolis alone had a significantly increase in WBCs count when compared with the control group. Propolis immunomodulatory action was thought to be limited mainly to macrophages, with no influence on lymphocyte proliferation [36]. Also, the water and ethanolic-extracts of propolis increased the percentage of phagocytes (monocyte, macrophages and acidophilic granulocytes) of gilthead seabream [11].

In the present study, propolis administration for non-infected fish (Group 3) induced non-significant changes in liver and kidney function when compared with negative control (Group 1) (Table 2). Propolis was safe and have no any side effects on serum biochemical parameters of rainbow [37], and female rats [38]. Fish experimentally infected with *A. hydrophila* and non-treated (Group 2) showed a significant increase in the liver and kidney functions (AST, ALT, urea and creatinine) except total protein was reduced at the two experimental periods (1^{st} and 10^{th} day post treatment) when compared with Group 1. Similar results were recorded by Amer *et al*. [30] who reported an increase in serum enzymatic activities in fish due to *A. hydrophila* infection.

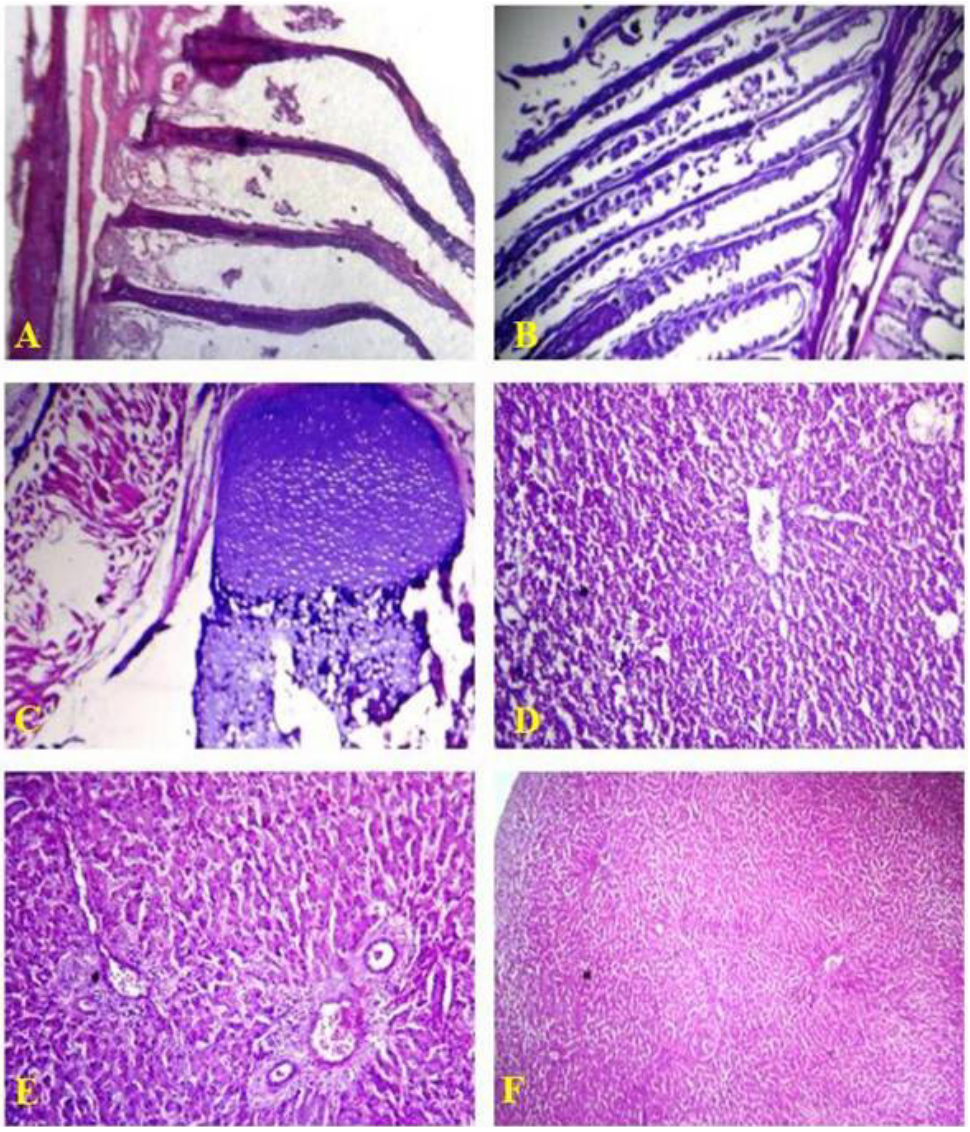

Figure 1: Histopathological changes of clinically healthy and infected *Clarius garpenius* with *A. hydrophila*. A: Section of gills of Group (2) showed deformation of primary lamellae (long arrow) with complete absence of the lining cells of the secondary lamellae (short arrow) (H&E x 400). B: Section of gills of Group (4) showed destruction of some lining cells of the secondary lamellae (H&E x 200). C: Section of gills of Group (4) showed necrosis of chondrocytes from cartilegenous part of the gill arch (H&E x 400). D: Section of liver of Group (2) showed mild congestion and vacuolation (H&E x 200). E: Section of liver of Group (2) showed mild congestion (short arrow) and few perivacular leucocytic infiltrations (long arrow) (H&E x 200). F: Section of liver of Group (6) showed normal structure of liver (H&E x 100).

The infected groups received propolis only (Group 5) or propolis with norfloxacin (Group 6) showed an improvement in total protein, globulin, AST, ALT, urea and creatinine levels at 1st and 10th day post treatment when compared with infected non treated group (Table 2). These improvements in biochemical parameters might be due to drug bactericidal effect [39] or the potential use of propolis as hepatoprotective agent and immune stimulant [34]. The treated group with norfloxacin (Group 4) showed a slight elevation in liver and kidney function at the two experimental periods when compared with Group 1 (Table 2). The same findings were reported by Amer *et al*. [30] who found that ciprofloxacin produced elevation in both urea and creatinine levels of fish.

The malondialdhyde (MAD) level revealed a significant decrease in Groups 4, 5 and 6 when compared with infected non-treated group (Table 2). Fish infected with *A. hydrophila* showed a significant increase in malondialdehyde activity in *Oreochromis niloticus* [40]. The significant decrease in MDA in Groups 5 and 6 might be related to flavonoids, which responsible for the antioxidant activity of propolis [35]. Propolis ameliorated the elevation in MDA of *Cyprinus carpio* exposed to chromium [41] and had antioxidant effects [3]. Our investigation showed a significant increase in the immunological parameters (phagocytic ratio and phagocytic index) in infected and non-infected groups treated with propolis (Group 3) and (Group 5) also, in combination with norfloxacin (Group 6) (Table 2). Several researchers suggested that propolis modulates the non-specific immunity via macrophage activation and stimulated cytokines production, such as IL-1β and TNFα, by peritoneal macrophages of mice. Moreover, they also able to modulate both in vivo and in vitro production of cytokines by macrophages as well as the complement receptor function either directly or via cytokines [36,42]. The immunodulatory action of propolis was mainly due to the macrophages with no influence on lymphocyte proliferation [43].

Gross examination of Group 2 revealed congestion of all internal organs and gills, while in the treated groups, they revealed mild macroscopical changes. Microscopically, gills of Group 2 showed deformation of primary lamellae with complete absence of secondary lamellae (Figure 1-A). Our results of group two are parallel to the results obtained by others [10,44] in infected catfish to *A. hydrophila* with high temperature and attributed to the gills are the target organ for *A. hydrophila* infection. Gills of Group 4 revealed abnormalities of secondary lamellae and destruction of others with necrosis of chondrocyte from cartilaginous part of the gill arch (Figures 1-B&C). These lesions were not detectable in other treated groups.

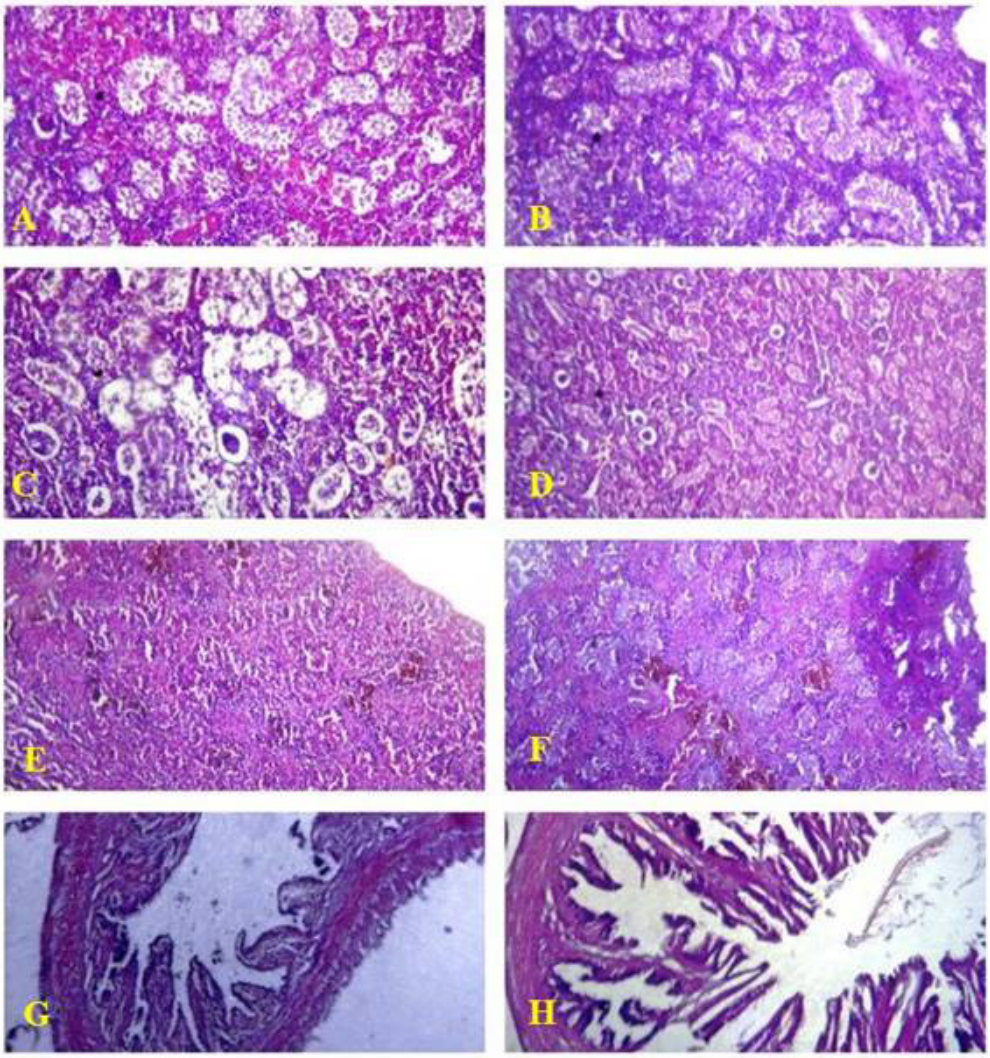

Figure 2: Histopathological changes of clinically healthy and infected *Clarius garpenius* with *A. hydrophila* and treated with norfloxacin and/or propolis. A: Section of kidneys of Group (2) showed diffuse degeneration of tubular epithelium in the renal cortex arrow head with hemorrhage (long arrow) and leucocytic infiltrations (short arrow) (H&E x 200). B: Section of kidneys of Group (2) showed coagulative necrosis (H&E x 200). C: Section of kidneys of Group (4) showed focal destruction of some renal tubules in the renal cortex and atrophy of some glomeruli (H&E x 200). D: Section of kidneys of Group (6) showed minimal degenerative changes (H&E x 100). E: Section of spleen of Group (2) showed haemosiderosis (H&E x 100). F: Section of spleen of Group (2) showed haemosiderosis (H&E x 100). G: Section of intestine of Group (2) showed fusion of some villi (long arrow) and necrosis of lining epithelium of others (short arrow) (H&E x 100). H: Section of intestine of Group (4) showed sloughing of epithelial lining some villi. (H&E x 200).

Liver showed severe congestion and vacuolation of hepatic cells in the hepatic parenchyma with few perivascular leucocytic infiltrations in infected non-treated fish with *A. hydrophila* (Group 2) (Figures 1-D &E). Otherwise, congestion of the hepatic blood vessels of infected fish treated with norfloxacin (Group 4) and propolis (Group 5) were recorded. While, apparently normal sections in portal area of infected fish liver treated with norfloxacin and propolis (Group 6) were detected (Figure 1-F). These results are in line with Mohamed [10] who reported an absence of histopathological changes in livers of catfish treated with propolis that attributed to its role as hepatoprotective agent.

Kidneys of the second group revealed a diffuse hemorrhage in renal cortex with a diffuse degeneration of tubular epithelium with leucocytic cell infiltrations with atrophy of some glomeruli in renal cortex and coagulatve necrosis (Figures 2-A &B). Otherwise, kidneys of Groups 4 and 5 showed moderate destruction of epithelial lining of some renal tubules with atrophy of some glomeruli in renal cortex (Figure 2-C). These lesions were not detected in Group 6, where normal renal tissue structure represented in minimal degenerative changes (Figure 2-D). Our results are similar to that obtained by Mohamed [10] who reported that normal structure of renal tissue of catfish treated with propolis is attributed to the synergistic effect of florfincol with propolis. Heart showed edema between cardiac muscle fibers in Group 2, which are similar to the results obtained by others [10,44], but there were no detectable lesions in other groups. Spleen showed haemosiderosis in the second group (Figures 2-E&F), while no detectable lesions in other groups. Intestine revealed submucosal edema, leucocytic cells infiltrations and fusion of some villi and necrosis of lining epithelium of others in Group 2 (Figure 2-G). Our results are similar to that obtained by Samnejhad *et al.* [44]. Sloughing of the epithelial lining of some villi was recorded in Group 4 (Figure 2-H), but no detectable lesions were recorded in other groups.

Conclusion

The results from this study suggested that administration of propolis alone or with a combination of antibiotic can ameliorate the harmful effects of *Aeromonas* infection in cat fish through their improvement of the hematological and biochemical parameters as well as the histopathological lesions.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربى

دراسات باثولوجيه وبيوكيميائيه على بعض مضادات الميكروبات في أسماك القرموط النيلي المعدي بالإيروموناس هيدروفيلا

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هذه الدراسة إستهدفت التحقق من كفاءة كل من صمغ العسل (البروبليز) والنورفلوكساسين ضد الإيروموناس هيدروفيلا في سمك القرموط النيلي. تم جمع السمك من مزرعة اسماك خاصة بمحافظة الشرقية وتم تغذيته على عليقة السمك. ثم قسمت الاسماك إلى ست مجموعات المجموعة الأولى غير معداه وغير معالجه، والمجموعة الثانية معداه معمليا بميكروب الإيروموناس هيدروفيلا وغير معالجه، المجموعه الثالثه غير معداه وتم إعطائها ١٠ جرام/ كيلوجرام وزن السمك لمدة عشر أيام بروبليز، المجموعه الرابعه معداه ومعالجه بالجرعة العلاجية من النورفلوكساسين، المجموعه الخامسه معداه ومعالجه بالبروبليز والمجموعه السادسه معداه ومعالجه بالجرعة العلاجية من البروبليز والنورفلوكساسين بالجرعات السابقة. وقد اوضحت النتائج ان البروبليز والنورفلوكساسين اكثر فاعلية ضد ميكروب الإيروموناس هيدروفيلا . ايضا القياسات الدموية تحسنت فى المجموعة الرابعة والخامسة والسادسة عند مقارنتها بالمجموعة الثانية. المجموعة الثانية اظهرت زيادة معنوية فى كل من الاسبرتات امينوترانسفيريز, الالانين امينوترانسفيريز, اليوريا ,الكيرياتينين والمالون داى الدهايد بينما هذه القياسات تحسنت بانخفاضها فى المجموعة الخامسة والسادسة .ايضا نتائجنا اوضحت زيادة معنوية فى القياسات المناعية فى المجموعات الثالثة والخامسة والسادسة. واكثر من ذلك هذه الدراسة ايضا اوضحت تغيرات باثولوجية فى الخياشيم والكبد والقلب والكلى والطحال والامعاء فى الاسماك المعداة بمكيروب الإيروموناس هيدروفيلا والتى اصبحت طفيفة فى الاسماك المعالجة خاصة مع البروبليز والمضاد الحيوى. هذه الدراسه اقترحت أن اعطاء كلا من البروبليز والنورفلوكساسين كان له كفاءة جيدة ضد الإيروموناس هيدروفيلا بدون اثار سلبية على القياسات الدموية والبيوكيميائية.