BIOCHEMICAL STUDIES ON THE EFFECT OF OFLOXACIN IN CAT FISH (*CLARIAS GARIPENUS*)

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Abstract

The present work was conducted to study the separate or combined effects of *Pseudomonas fluorescens* infection and its treatment with ofloxacin on mortality rate, body weight and some biochemical parameters in catfish. One hundred catfish were divided into 4 groups. The first group (25 fish) was used as a control. The second group (25 fish) was non-infected and treated with therapeutic dose of ofloxacin for 5 successive days. The third group (25 fish) was infected with *Pseudomonas fluorescens* and non-treated. The fourth group (25 fish) was infected and treated. Blood samples were collected one day, one week and two weeks post-treatment in groups 2 and 4 and post-appearance of clinical signs in group 3.

Ofloxacin alone (group 2) significantly increased body weight, produced significant and reversible increase in serum ALT, AST and AP activities, as well as, glucose and creatinine levels. Pseudomonas infection alone (group 3) produced skin ulcers, 40% mortality, significantly decreased body weight, significant and reversible increase in ALT, AST and AP activities, as well as, glucose level. Treatment of infection (group 4) prevent mortality, returned body weight to control level, also produced significant and reversible increase in liver enzymes and glucose level. Ofloxacin can be used safely for treatment of Pseudomonas infection in catfi

INTRODUCTION

Fish is considered as an important and valuable source of animal protein of a highly nutritious value.

Pseudomonas fluorescens is a Gram-negative bacteria. It is considered as a fish spoilage organism and the etiological agent of pseudomonad septicemia which is a hemorrhagic disease of fish (Bullock and Mclaughlin, 1970). Moreover, it causes deaths due to production of toxic proteases (Fayed *et al.*, 1997). The infection may be acute or chronic.

Fluoroquinolones are a new class of bactericidal antimicrobial agents, having tremendous potential for use in Veterinary Medicine because of their broad spectrum activity against a wide range of Gram-negative and Gram-positive bacteria. They are active at low concentration and of low toxicity (Vancutsem *et al.*, 1990).

Fluoroqinolones include ofloxacin, ciprofloxacin, norfloxacin, acrosoxacin, pefloxacin, danofloxacin, enrofloxacin and nalidixic acid. The bactericidal activity of fluoroquinolones is due to inhibition of bacterial DNA gyraze enzyme (Laurence *et al.*, 1997). Fluoroquinolones have several attractive pharmacokinetic properties that enable their oral administration for the treatment of infection that formerly required parenteral therapy (Brenner, 2000).

Ofloxacin is a synthetic antibacterial agent of the fluoroquinolones class that was developed for Veterinary use. It is a new pyridone carboxylic acid derivative of nalidixic acid, chemically known as 9 Fluoro-2, 3 dihydro-3 methyl-10 (4-methyl piprazinyl) 7 oxa-7H-pyrido [1,2,3-de]-1,4 benzoxazine-6-carboxilic acid. The drug is concentrated in many tissues, particularly in the kidney. Excretion is largely renal with some biliary excretion (Craig and Stitzel, 1994).

Fluoroquinolones have been associated with hypoglycemia and nephrotoxicity on some occasions (Craig and Stitzel, 1994).

The present work was designed to study the effect of ofloxacin in non-infected and experimentally infected catfish.

MATERIALS AND METHODS

Fish: A total of 100 clinically healthy catfish (*Clarias garipenux)* of both sexes was used. Fish were kept in glass aquaria (30x80x20) of 50 liters capacity containing dechlorinated tap water by adding sodium thiosulphate and fed on catfish ration. Fish were acclimatized for 7 days before the experimental work, and supplied with O2 by air compressor, T.(24+1) adjusting with thermostat.

Ofloxacin: (ofloxacin-20)** 20% solution was given in water by therapeutic dose (50mg/litre) for 5 successive days.

Bacterial organism: A strain of *Pseudomonas fluorescens**** was used. Infected groups were injected I/M with 0.3ml virulent isolate of *Ps-fluorescens* (3 x 10^6 living bacterial cell/ml of saline suspension) obtained from Microbe Enrichment Center (MERCEN).-Faculty of Agricultur-Ein Shams Univ. The opacity of bacterial suspension was adjusted against standard Mac Ferland tubeNo.3 to estimate the number of bacteria in each ml of suspension according to *Cruickshank et al. (1975).*

Infected groups were observed for appearance of clinical signs.

Experimental work: The experimental work was summarized in Table 1.

Table 1. Summary of the experimental work.

Groups	No. of fish in each group	Infection with Ps. Fluorescens	Treatment with ofloxacin
G_1 (Control)	25	Non infected	Non treated
G_2	25	Non infected	Treated
G_3	25	Infected	Non treated
G_4	25	Infected	Treated

Blood samples were collected from caudal vein from five fish in each group after one day, one week and two weeks post-treatment in G_2 and G_4 and in the same periods post-appearance of clinical signs in G_3. Serum was separated and used for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957), alkaline phosphatase (AP), total protein , albumin according to Henry (1946) glucose, uric acid according to Trived *et al.* (1978) and creatinine according to Bartels (1971).

** (Ofloxacin-20) was obtained from Samu Chemical Ind Co., LTD, Deungchon-Dong Kangseo-ku, Seoul, Korea.

***_Pseudomonas fluorescens_ was obtained from Microbe Enrichment Center (MERCEN)-Faculty of Agriculture-Ein Shams Univ.

Also, mortality rate was recorded in each group. In addition , all fishes were weighed individually at the beginning and at the end of the experiment.

Results of the tested parameters were statistically analyzed (F-test) according to Tamhane and Dunlop (2000) using MSTAT-C computer program. Means in the same columns followed by different letters were statistically significant and the highest value was represented with the letter a.

RESULTS AND DISCUSSION

Infection of fish with virulent isolate of *Ps. fluorescens* resulted in appearance of clinical signs 24 h post-infection in the form of patches of skin discolouration and exophthalmia. Three days post-infection, skin ulcers appeared on the abdomen, petechial hemorrhages on the gills in some cases and abdominal swelling in other cases. Infected dead fishes showed congestion and enlargement of the parenchymatous organs. The same results were previously reported by Zeinab (1986) and Saad *et al.* (1998). Mortality rate was found only in group 3 (infected non-treated) by the ratio of 40%. As shown in Table 2, mortality rate due to infection with *Ps. fluorescens* was previously reported by Zeinab (1986) and Saad *et al.* (1998). No mortalities were recorded in infected treated group (G_4). This might be attributed to the broad spectrum activity of the drug against many pathogens (Vancutsem *et al.,* 1990).

Table 3 shows final body weight in all experimental groups. Compared with the control group, G_2 (non-infected and treated) showed the highest body weight, while G_3 (infected non-treated), showed the lowest body weight. The increase in body weight due to ofloxacin therapy was previously reported by Alexander (1985). He mentioned that the increase in body weight might be attributed to the effect of the drug on to subclinical infections, specially the intestine, promoting an increase absorption of nutrients and, consequently improvement of general health conditions.

Table 4 shows changes in some liver enzymes in experimental groups compared with the control. ALT showed highly significant increase in groups 2, 3 and 4 after 1^{st} day. The highest value was obtained in (G_2) by the ratio of 200%. AST showed highly significant increase on the 1^{st} day, the highest values were obtained in groups 2 and 4 by the ratios of 149.21% and 146.03%, respectively. ALT and AST

showed a significant increase after the 1st week which was similar in all experimental groups. ALT and AST showed non-significant changes after the 2nd week indicating that the effect of the drug or the microbe was transitory.

Alkaline phosphatase showed highly significant increase in all groups on the 1st day and in the 1st week which was similar in all groups, and non-significant change in the 2nd week indicating that the effect of the drug or the microbe was transitory. Elevated levels of ALT, AST and AP may be due to transitory disturbance in liver functions produced either by the microbe or the drug. The increase in these enzymes due to ofloxacin therapy was previously reported by Laurance *et al.*(1997). They reported that administration of ciprofloxacin and enrofloxacin to catfish produced an increase of serum AST and ALT enzymes.

The increase in these enzymes in infected group might be due to the effect of *Ps. fluorescens* on the liver. Zeinab (1986) mentioned that in case of *Ps. fluorescens* in catfish, liver was enlarged with minute hemorrhage and multiple necrotic foci.

Table 5 shows non significant changes in total protein, albumin, globulin and A/G ratio in all groups compared with the control group in all periods. Non-significant change in albumin in groups 3 and 4 either due to *Ps. fluorescens* infection alone or infection and treatment with norfloxacin was previously reported by Saad *et al.* (1998) in catfish.

Table 6 showed highly significant decrease in glucose level after the 1st day and 1st week in groups 2, 3 and 4 compared with control group, and non significant decrease in glucose level in all groups after the 2nd week. The hypoglycemic effect due to the drug or the microbe may be attributed to transitory disturbance in liver function. Hypoglycemia due to drug administration was previously reported by *Craig and Ctitzel* (1994).

Table 6 shows non significant changes in serum uric acid in all groups in all periods compared with the control group. Uric acid is formed by fish from exogenous and endogenous purine nucleotides and by catabolism of proteins via purines. It is converted in the liver, and to a lesser extent in the kidney, to urea for excretion by the gills (Stoskopf, 1993). Normal values of serum uric acid in this work may be attributed to liver dysfunction is not severe enough to alterate uric acid level in the blood. Non-significant change in uric acid due to drug administration (Table 6) shows a significant increase in creatinine level in group 2 only compared with the control group by the

ratio of 40% after the 1st day, non-significant changes in creatinine level in all groups after the 1st and 2nd weeks. A transitory increase in serum creatinine due to the effect of the drug may be due to mild reversible kidney dysfunction (Lockart and Menter 1984). The increase in serum creatinine level due to the effect of the drug was previously reported by Kobayashi (1985).

Table 2. Mortality rate in the experimental groups.

Groups	Initial no. of fish	No. of dead fish	%
G_1	25	-	Zero
G_2	25	-	Zero
G_3	25	10	40
G_4	25	-	Zero

Table 3. Body weight (mean values ± SE) in experimental groups.

Groups	Initial body weight (gm)	Final body weight (gm)
G_1	150 ± 1.25	180 ± 2.89 b
G_2	148 ± 1.00	225 ± 3.39 a
G_3	150 ± 1.25	160 ± 2.89 c
G_4	152 ± 1.00	175 ± 3.39 b
F-test	N.S	**
L.S.D.	–	9.41

L.S.D.: Least significant difference.

N.S: Non significant.

** : $P < 0.01$.

Table 4. Changes in some liver enzymes of catfish in the experimental groups at all periods.

(mean ± SE) n=5

Parameters	ALT (U / 1)			AST (U / l)			AP (U / l)		
Periods / Groups	1st day	1st week	2nd week	1st day	1st week	2nd week	1st day	1st week	2nd week
G_1	13.0±0.58c	13.2±0.88b	12.3±0.88	31.5±0.87c	31.3±0.67b	32.0±0.58	15.8±0.58b	15.5±0.58b	15.3±0.88
G_2	26.0±1.16a	15.8±0.58a	13.0±0.58	47.0±1.16a	37.3±2.19a	34.0±0.58	27.4±0.55a	26.7±0.40a	15.3±0.88
G_3	19.0±1.53b	16.3±0.33a	15.0±1.53	37.5±0.87b	37.0±0.58a	33.0±0.88	28.4±0.44a	28.2±0.30a	16.9±0.52
G_4	21.7±0.67b	16.3±0.33a	15.7±1.20	46.0±1.16a	40.0±0.58a	34.7±0.67	28.4±0.61a	27.0±0.41a	18.7±1.97
F-test	**	*	N.S	**	*	N.S	**	**	N.S
L.S.D	3.44	1.88	—	3.33	3.50	—	1.78	2.41	—

L.S.D.: Least significant difference.

N.S: Non significant.

* $P < 0.05$.

** $P < 0.01$.

Table 5. Proteinogram (mean values ± S.E) of catfish in the experimental groups at all periods. n=5

Parameters	Total protein (gm/dl)			Albumin (gm/dl)			Globulin (gm/dl)			A/G Ratio		
Periods / Group	1st day	1st week	2nd week	1st day	1st week	2nd week	1st day	1st week	2nd week	1st day	1st week	2nd week
G_1	3.77±0.32	4.22±0.24	4.50±0.59	1.34±0.02	1.33±0.03	1.35±0.06	2.43±0.34	2.89±0.24	3.15±0.64	0.57±0.09	0.47±0.04	0.47± 0.10
G2	4.11±0.12	4.15±0.08	4.44±0.39	1.36±0.04	1.35±0.03	1.36±0.01	2.75±0.15	2.80±0.10	3.08±0.39	0.50±0.04	0.48±0.03	0.46+0.06
G3	4.27±0.59	3.98±0.08	4.08±0.15	1.53±0.12	1.40±0.12	1.39±0.23	2.74±0.51	2.58±0.16	2.69±0.08	0.57±0.09	0.55±0.07	0.52+0.10
G4	4.23±0.12	4.22±0.14	4.34±0.21	1.52±0.26	1.45±0.13	1.50±0.14	2.71±0.34	2.77±0.22	2.84±0.21	0.56±0.14	0.52±0.06	0.54+0.08
F-test	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
L.S.D.	—	—	—	—	—	—	—	—	—	—	—	—

L.S.D.: Least significant difference.

N.S: Non significant.

Table 6. Changes in glucose, uric acid and creatinine of catfish in the experimental group at periods.

(mean ± SE)

n=5

Parameters	Glucose (mg/dl)			Uric acid (mg/dl)			Creatinine (mg/dl)		
Periods / Groups	1st day	1st week	2nd week	1st day	1st week	2nd week	1st day	1st week	2nd week
G_1	59.7±0.78a	60.0±2.21a	59.3±0.58	1.77±0.21	1.54±0.32	1.68±0.10	0.50±0.05b	0.54±0.05	0.47±0.10
G_2	39.1±2.68b	47.9±2.07b	59.1±2.82	1.68±0.05	1.63±0.06	1.65±0.03	0.70±0.03a	0.50±0.02	0.51±0.03
G_3	42.2±2.44b	46.3±1.47b	52.4±2.88	1.57±0.09	1.55±0.04	1.72±0.11	0.42±0.05b	0.45±0.04	0.42±0.02
G_4	43.7±2.86b	51.0±2.60b	53.3±4.09	1.69±0.14	1.61±0.02	1.62±0.03	0.42±0.11b	0.41±0.11	0.42±0.11
T-test	**	**	N.S	N.S.	N.S	N.S	*	N.S	N.S
L.S.D	7.64	7.93	—	—	—	—	0.18	—	—

L.S.D.: Least significant difference.

N.S: Non significant.

* $P < 0.05$.

** $P < 0.01$.

In conclusion

- Ofloxacin increased body weight produced significant and reversible changes in liver enzymes, glucose and creatinine level in catfish.
- *Ps. fluorescens* induced severe mortality in catfish, decreased body weight, produced skin ulcers and significant reversible changes in liver enzymes and glucose.
- Ofloxacin can be used safely in catfish for treatment of *Ps. fluorescens* infection to prevent mortalities and improve general health conditions.

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دراسات بيوكيميائية على تأثير عقار الأفلوكساسين على سمك القرموط (كلارياس جاربينوس)

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إستهدف هذا البحث دراسة تأثير كل من العدوى بميكروب السودوموناس والعلاج بعقار الأوفلوكساسين كل على حدة أو كليهما معاً على معدل النفوق ووزن الجسم وبعض الدراسات البيوكيميائية فى اسماك القرموط. أستخدم فى هذا البحث مائة سمكة قسمت إلى أربع مجموعات المجموعة الأولى (٢٥ سمكة) وهى المجموعة الضابطة. المجموعة الثانية (٢٥ سمكة) غير معدية ومعالجة بالجرعة العلاجية للأوفلوكساسين لمدة خمسة أيام. المجموعة الثالثة (٢٥ سمكة) تم عدواها بميكروب السودموناس وتركت بدون علاج. المجموعة الرابعة (٢٥ سمكة) تم عدواها وعلاجها. تم أخذ عينات الدم بعد يوم وأسبوع وأسبوعين من نهاية العلاج فى المجوعة الثانية والرابعة ومن ظهور الأعراض فى المجوعة الثالثة.

وأظهرت النتائج زيادة معنوية في وزن الجسم وإنزيمات الكبد والجلوكوز والكرياتينين فى المجموعة الثانية. والتغيرات فى السيرم كانت مؤقته وعادت للطبيعي بعد إيقاف العلاج بأسبوعين أما العدوى بالسودوموناس (المجموعة الثالثة) فأدت إلى معدل نفوق ٤٠%، وظهور قرح بالجلد وقل وزن الجسم معنوياً بالإضافة إلى زيادة معنوية ومؤقتة في إنزيمات الكبد والجلوكوز. أما المجموعة الرابعة فقد أدى علاج العدوى إلى عدم حدوث نفوق وعودة وزن الجسم إلى مستوى المجموعة الضابطة وزيادة معنوية ومؤقتة في إنزيمات الكبد والجلوكوز.ويستنتج من هذه النتائج أنه يمكن استخدام عقار الأوفلوكساسين بأمان لعلاج عدوى السودوموناس في سمك القرموط النيلي.