

EFFICACY OF SULPHATRIM AND AMOXI-VET AGAINST AEROMONAS HYDROPHILA INFECTION IN NILE TILAPIA

Reham, A. El-Shafet; Mohamed G. El-Sayed and Magdy, M. Amer

Pharmacology Department, Faculty of Veterinary Medicine,

Mansoura University, Mansoura, Egypt

ABSTRACT

To determine the efficacy of Sulphatrim and Amoxi-vet against *Aeromonas hydrophila* infection in Nile tilapia fish and their effect on fertility of fish, a total number of 144 fish were divided into 6 equal groups (each of 24 fish). Then each group divided to A and B subgroups. The sensitivity test and MIC of the tested drugs were determined against *Aeromonas hydrophila* M. O. Mortality rate and the observed clinical signs were also recorded. Milt picture (sperm count, motility and vitality %) were determined as well as some reproductive hormones (estrogen, progesterone and testosterone) were measured. It was concluded that Sulphatrim has more powerful efficacy *in vitro* and *in vivo* against *A. hydrophila* than amoxicillin, as well as has less adverse effects on the milt picture and on the tested reproductive hormone in male and female Nile tilapia fish. However it was found that Amoxi-vet causes decrease in sperm count as well as sperm motility and vitality and also the tested reproductive hormones.

INTRODUCTION

Aquaculture is considered to be the only possible solution to increase fish production in Egypt. Owing to the increasing importance of aquaculture to compensate the progressive worldwide reduction of natural fish and to the fact that several fish farming often suffer from heavy financial losses due to the development of infections caused by microbial pathogens. However, some species of bacteria such as *Streptococcus*, *Aeromonas* and *Edwardsiella* can easily infect tilapia during some stages of culture having profound impacts on productivity, it is necessary to be treated with antibacterials to reduce losses due to bacterial infections (Shocmaker and Klestus, 1997).

During the past decade considerable progress has been made in the therapy of bacterial fish diseases. This progress was possible largely because of the introduction of powerful chemotherapeutic agents. Two commonly used classes of antibacterials are sulphonamides and B-lactams (Mark et al., 1997).

Nile tilapia, (*Oreochromis niloticus*) is an important species for freshwater aquaculture and considered to be one of the most important warm-water cultured fish species in the world (Plumb, 1999). Rurangwa et al (2004) stated that fish farming industry has been more focused on the quality of eggs or larvae as well as that of sperm. Sperm quality

affected mainly by the factor of motility as the high motility of sperm is a prerequisite for fertilization and correlates strongly with fertilization success.

Aim of the work : No data are published about the effect of Sulphatrim and Amoxi-vet in fish fertility so the objectives of our study were to:

- 1- Investigate the efficacy of Sulphatrim and Amoxi-vet in treating experimentally infected *Oreochromis niloticus* with *Aeromonas hydrophila*.
- 2- Determine how much these drugs affect fertility and reproductive activities of male and female *Oreochromis niloticus*.
- 3- Study the effect of the tested drugs on some biochemical parameters of *Oreochromis niloticus*.

MATERIAL AND METHODS

Drugs:

Sulphatrim®: Available as tablets, each tablet contains: Sulphadiazine 1gm and Trimethoprim 0.2gm Produced by Unipharm Company. Sole agent, Al-Abrar Co., Cairo, Egypt.

Amoxi-vet®: Available as tablets, each tablet contains Amoxicillin Trihydrate equivalent to 500 mg Amoxicillin anhydrous. Obtained from Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt.

Fish: A total number of 144 apparently healthy Nile tilapia obtained from fish farm in Faculty of Agriculture, Mansoura University with average body weight of 100 ± 50 gm transported alive to the laboratory of Pharma-

cology, Faculty of Veterinary Medicine, Mansoura University.

Aquaria : Fourteen glass aquaria measuring 40 x 70 x 100 cm³ were used and provided by aerating devices, heaters and supplied with fresh dechlorinated tap water according to **Innes (1986)**.

Biological agents :

Bacterial strains (*Aeromonas hydrophila*):- The identified *A. hydrophila* isolate used in the experimental infection was obtained from Department of Fish Diseases, Faculty of Veterinary Medicine, Mansoura University.

Biochemical kits:- Kits used for determination of serum transaminases , serum urea level, serum creatinine level, serum total protein and serum albumin were purchased from (Diamond Diagnostic, Egypt).

Stains : Nigrosin-Eosin stain.

Experimental design :

Fish grouping : To determine the efficacy of Sulphatrim and Amoxi-vet against ***Aeromonas hydrophila*** infection in **Nile tilapia** fish and the effect of the two examined drugs on the fertility of fish, one hundred and forty four fish were divided into 6 equal group each of 24 fish.

Blood samples : Blood samples were collected directly from the caudal veins of five fish of each group at 1st, 7th and 14th day post medication. Blood was taken in clean dry centrifuge tubes and allowed to clot at room temperature for 2 hours. Then centrifuged at 3000 rpm for 15 minutes. Separated sera were collected in 1.5 ml Eppendorf

tubes, labeled and kept frozen at -20 till analyzed.

Milt samples: Milt samples were obtained by genital pressure in the abdominal region of five male fish of each group at 1st, 7th and 14th day post medication of all male groups then examined microscopically.

Efficacy of Sulphatrim and Amoxi-vet against *Aeromonas hydrophila*.

In-vitro - Sensitivity test (disc diffusion method).

The in-vitro antibacterial effect of Sulphatrim and Amoxi-vet against *Aeromonas hydrophila* was carried out using disc diffusion method (Bauer et al., 1966). The technique was standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 1994). The interpretation of inhibition zones was estimated according to the limits given by (Quinn et al., 1994) to determine whether the organism is sensitive or resistant to the tested drug.

In-vivo : Effect of Sulphatrim and Amoxi-vet on pathogenesis and mortality rate % of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. All fish groups were observed daily, mortalities and any adverse signs were recorded.

Effect of Sulphatrim and Amoxi-vet on fertility of clinically healthy and experimentally infected male and female *Oreochromis niloticus* with *A. hydrophila*.

Microscopical examination of the collected milt for determination of sperm count, motility and vitality.

Determination of Sperm count using hemocytometer : Sperms of male fish groups were counted using hemocytometer slide according the method of (Becton, 1977).

Determination of Sperm motility: Sperm motility of fish was determined by placing 5µL of fresh milt on clean dry glass slide, diluted with 50 µL distilled water then covered with cover slip, and examined under microscope using high power lens (40 x). The sperm motility was expressed as the percentage of spermatozoa showing a linear progressive forward motility.

Determination of Sperm Viability: The World Health Organization (1992) method for assessing the viability of the spermatozoa was used. A 50 µl aliquot of sperm suspension and 50 µl of the Nigrosin-Eosin stain were thoroughly mixed in a microfuge tube and gently smeared onto ethanol-cleaned microscope slide. The slide was allowed to air dry for 30 min and examined by microscope using oil immersion lens (100 x). The percentage of viable cells, that excluded the dye, was calculated from assessment of 100 cells.

Effect on the reproductive hormones: Estrogen, Progesterone and Testosterone hormones were determined in the serum samples at the Department of Clinical Pathology, Faculty of Medicine, Mansoura University.

Effect of Sulphatrim and Amoxi-vet on liver, kidney function tests and serum proteins.

Determination of serum transaminases : The serum levels of Alanine aminotransferase (ALT) and serum Aspartate aminotransferase

(AST) were determined colorimetrically according to the method of **Reitman and Frankel (1957)**.

Determination of serum urea level : Determination of serum urea level was determined calorimetrically according to the method of **Patton and Crouch (1977)**.

Determination of serum creatinine : Serum creatinine level was determined calorimetrically according to the method of **Henry (1974)**.

Determination of serum total proteins : Serum total proteins were measured calorimetrically chemically according to the method of **Doumas (1975)**.

Determination of serum albumin : Albumin was determined according to **Doumas et al. (1981)**.

Calculation of Serum globulin: Serum globulin was determined by subtraction of the obtained albumin level from the level of total proteins as described by **Doumas and Biggs (1972)**.

Statistical analysis : Data obtained in this study were statistically analysed for variance (ANOVA), and least significant difference (LSD) as described by **Snedecor and Cochran, (1967)** by using computerized **SPSS (1996)** version 10.0.

RESULTS AND DISCUSSION

These studies were carried out to reveal the efficacy of Sulphatrim and Amoxi-vet against experimentally infected *Oreochromis niloticus*

with *Aeromonas hydrophila* pathogen. Special attentions were directed to determine the effect of these drugs on the fertility of fish. In addition, some biochemical parameters were also determined.

In-vitro sensitivity test of Sulphatrim and Amoxi-vet against *A. hydrophila* strain using agar disc diffusion method indicated its high sensitivity to Sulphadiazine-trimethoprim combination. Similar result were obtained by **Ceylanac et al., (2003)** who found that *A. hydrophila* was sensitive to sulfamethoxazole-trimethoprim. Also our results showed that *A. hydrophila* was sensitive to amoxicillin. A result which is similar to that of **Jindal et al., (1993)** who reported that *A. hydrophila* was sensitive to amoxicillin.

The in-vivo effect of Sulphatrim and Amoxi-vet was evaluated against experimentally infected *O. niloticus* with *A. hydrophila* organism. Our results revealed that experimentally infected *O. niloticus* with *A. hydrophila* organism responded to the treatment with sulphadiazine-trimethoprim, as the recorded clinical signs were declined within 5 days post treatment. Sulphadiazine-trimethoprim also combination appears to control mortalities associated with *Aeromonas hydrophila* in fish. These results were in agreement with that of **Iain et al., (2007)** who mentioned that potentiated sulphonamides are used in control of many bacterial diseases of fish. While the efficacy of Amoxi-vet to treat *O. niloticus* experimentally infected with *A. hydrophila* was less than Sulphatrim as the clinical signs were declined after 10 days of the treatment. These results were coincides with **Miller and Richards (1993)** who said that combination of

clavulanic acid with amoxicillin was not effective in treating *Aeromonas salmonicida* in farmed Atlantic salmon. However these results were in contrast with those of **Ingle et al., (1992)** who investigate the efficacy of amoxicillin in the control of laboratory induced *Aeromonas salmonicida* infection in Atlantic salmon, they found that when amoxicillin given in the diet (80 mg / kg bodyweight) was effective against severe challenge where mortality rates in untreated groups of 75 % reduced to 45 % in treated ones.

Regarding the effect on fertility we found that all tested reproductive parameters, milt picture and the level of both male and female sex hormones has a significant decrease in infected non treated group in comparison with those of non infected non treated control group. This was in full agreement with **Foo and Lam, (1993)** who reported that stress was often blamed for the suppression of gonadal development, production of sex steroids and gonadotropin secretion in salmonid fishes. The recorded results revealed that the group treated with Sulphatrim treated group showed non significant reversible changes on milt picture as well as on the estimated sex hormones of both male and female fish. These results were nearly similar to those of **Park and Choi, (2008)** who reported that exposure to sulfathiazole, resulted in greater expression of CYP₁₇, CYP₁₉, or 3HSD₂, which play crucial role in steroidogenic pathways, resulted in altering hormone production and aromatase activity in *Daphnia magna*.

While in amoxi-vet there were a significant decrease in the sperm count, sperm motility as well as the tested sex hormones in both

male and female treated tilapia when compared with control non infected non treated group. These finding are in accordance with those recorded by **Gracia et al., (2007)** who stated that antibiotics, such as amoxicillin, tylosin, and oxytetracycline, can modulate hormone production related to steroidogenesis.

Serum transferases (AST and ALT) activities are considered a sensitive indicator to evaluate hepatocellular and myocardial damage hence the synthesis of these enzymes is mostly of hepatic origin (**Abo-Hegab et al., 1992**).

Serum aminotransferase of *O. niloticus* were significantly influenced after *A. hydrophila* infection. Our results indicated that serum aminotransferases were significantly increased in infected non treated *O. niloticus* ($p < 0.05$) in comparison to control values. Our findings were confirmed by **Radcot et al. (1975)** who reported that there were increases in serum enzymatic activities of diseased fish suffering from bacterial infection. These changes appeared as a result of the escape of enzymes to the serum of injured hepatic cells due to bacterial infection. Further more, **Verma et al. (1981)** stated that, the increased serum transferases might be due to the hepatic damage which leads to liberation of these enzymes to the blood circulation.

In the present study we found that there were non significant changes in the level of AST and ALT enzyme activities following Sulphatrim administration. These results were nearly similar to that recorded by **Sanjay and Anil, (2008)** who mentioned that Sulphonamides has dose dependent hepatotoxicity. On

the other hand, fish infected with *A. hydrophila* and treated with Sulphatrim and Amoxi-vet elicited a moderate improvement in serum transferases when compared with the infected non treated group. These improvements in liver function might be due to the bactericidal effect of these drugs which limit the destructive and toxic effects of *A. hydrophila* on the liver as well as the regenerative process which takes place in the liver cells.

Concerning serum level of urea and creatinine, there were a significant increase in urea and creatinine levels in infected non treated group of fish compared with those of control fish, and these results were similar to those explained by **Castillas et al. (1983)** who mentioned that there were an elevation of urea and creatinine levels in fish with bacterial infection. This might be due to damage of kidney cells caused by bacterial infections.

Lockhart and Mentner, (1984) found that, elevation of creatinine level is an indicator of kidney dysfunction. While the increase in urea level in serum of fish is an indicator to gill dysfunction as urea is excreted mainly through gills. Moreover, **Wright and Land, (1998)** reviewed that there are several factors that increase excretion of urea in fish subjected to stressful conditions, such as high concentrations of environmental ammonia, high pH, exposure to infection or crowding.

Regarding the group treated with Sulphatrim, we found a significant elevation in both urea and creatinine levels and this result was supported by those reported by **Dijkmans et al., (1982)** who revealed that co-trimoxazole (sulphamethoxazole+trimethoprim) induce a

highly significant and reversible elevation of the serum creatinine level. Also these results were in accordance with the suggestion of **Berglund (1970)** who explained that co-trimoxazole may inhibit the tubular secretion of creatinine. However previous studies of **Hood et al., (1975)** revealed no effect of co-trimoxazole on the renal function in patients with a renal dysfunction. While in amoxicillin treated group, we found non significant decrease in serum urea and creatinine levels compared with those of control non infected non treated group. Our result were supported with the results of **Lee and Hill, (1968)** who reported that urea clearance was reduced to slightly below normal with ampicillin administration, also there were a changes in renal function and reduction in serum creatinine level. **London, (1967)** have reported the occurrence of severe haematuria and proteinuria following administration of methicillin and penicillin G.

On the other hand, fish infected with *A. hydrophila* and treated with Sulphatrim and Amoxi-vet showed a moderate significant improvement in serum urea and creatinine levels when compared with infected non treated groups. This improvement in kidney function might be due to the bactericidal effect of these drugs which limit the destructive and toxic effects of *A. hydrophila* on the kidney as well as the regenerative process which takes place in the kidney cells.

Regarding Effect on serum total protein and it's fraction **Riedmuller (1966)** observed that there was a decrease in serum total proteins in brown trout fish infected with *A. hydrophila*. This observed hypoproteinemia was attrib-

uted to hepatocyte disorders which results in increasing the catabolic rate. In addition **Tietz (1987)** reported that the abnormal fluctuation of serum proteins might be observed in acute inflammation of liver and nephrotic syndrome associated with bacterial infection.

The significant decrease in serum albumin in infected non treated group could be attrib-

uted to the destructive effect of *A. hydrophila* pathogen and its toxin on liver cells, thus impairs the synthesis of serum albumin, these results were in agreement with that of **Bayazit et al., (2004)** who showed that albumin levels tend to be decreased in severe infectious diseases. Also **Mottelib, (1972)** explained that bacterial infection might decrease the serum albumin.

Table (1): Interpretation chart for the size of growth inhibition zones according to Quinn et al. (1994).

Tested Drugs	Diameters of Inhibition Zones (mm)	Interpretation
Enerofloxacin (E-F) (10)	32	Susceptible
Peфлоxacin (PL) (10)	26	Susceptible
Amoxycillin (AMC) (30)	15	Moderately Susceptible
Sulphadiazin-Trimethoprim (S+T)	16	Susceptible
Neomycin (N) (30)	18	Susceptible
Thiamphenicol (TM) (30)	11	Susceptible

Table (2): The effect of Sulphatrim and Amoxi-vet on mortality rate (%) of experimentally infected *Oreochromis niloticus* inoculated with *A. hydrophila*.

Fish grouping	Total number	Number of dead fish	Mortality (%)
Non infected non treated (G1)	24	0	0%
Infected non treated (G2)	24	13	54%
Infected treated with Sulphatrim (G5)	24	4	16%
Infected treated with Amoxi-vet (G6)	24	6	23%

Table (3): The effect of Sulphatrim and Amoxi-vet on sperm Picture (Count , Motility and Vitality) of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. (M±S.E) (n=5).

Group	1 st day			7 th day			14 th day		
	Sperm count (/mL) X10 ⁷	Sperm Motility %	Sperm Viability %	Sperm count (/mL) X10 ⁶	Sperm Motility %	Sperm Viability %	Sperm count (/mL) X10 ⁸	Sperm Motility %	Sperm Viability %
G 1	2.32	91.0	88.60	2.32	91.40	88.60	2.44	90.60	88.80
	± 0.11 ^a	± 1.48 ^a	± 3.05 ^a	± 0.09 ^a	± 1.28 ^a	± 1.77 ^a	± 0.06 ^a	± 1.46 ^a	± 2.08 ^a
G 2	0.16	46.80	59.60	0.13	51.0	59.60	1.52	55.60	79.00
	± 0.01 ^d	± 1.31 ^c	± 3.26 ^d	± 0.01 ^d	± 1.87 ^d	± 2.03 ^b	± 0.13 ^d	± 1.69 ^d	± 2.42 ^c
G 3	2.18	85.00	82.0	2.26	87.20	82.00	2.40	87.60	89.40
	± 0.11 ^a	± 1.37 ^b	± 1.97 ^b	± 0.06 ^a	± 0.96 ^a	± 2.19 ^a	± 0.08 ^a	± 1.12 ^b	± 0.74 ^a
G 4	1.20	72.40	73.0	1.72	73.80	73.0	2.02	77.60	72.80
	± 0.05 ^b	± 1.12 ^c	± 1.22 ^c	± 0.04 ^b	± 0.73 ^c	± 0.63 ^b	± 0.05 ^b	± 1.12 ^c	± 1.39 ^d
G 5	0.96	69.0	73.40	1.72	79.20	73.40	2.28	83.60	85.20
	± 0.04 ^c	± 1.18 ^c	± 1.43 ^c	± 0.08 ^b	± 3.27 ^b	± 1.35 ^b	± 0.09 ^a	± 1.56 ^b	± 1.46 ^{ab}
G 6	0.84	62.40	73.80	1.34	70.80	73.80	1.96	76.0	83.60
	± 0.01 ^c	± 1.12 ^d	± 1.62 ^c	± 0.11 ^c	± 1.65 ^c	± 1.46 ^b	± 0.06 ^c	± 0.63 ^c	± 1.56 ^{bc}

Table (4): The effect of Sulphatrim and Amoxi-vet on the male testosterone (ug/dL) of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. (M±S.E) (n=5).

Fish Grouping	Testosterone (ug/dL) / Day Post Treatment		
	1 st day	7 th day	14 th day
G 1	2.63±0.04 ^a	2.64±0.04 ^a	2.64±0.05 ^a
G 2	1.07±0.02 ^d	1.09±0.01 ^e	1.22±0.01 ^c
G 3	1.88±0.04 ^c	1.65±0.07 ^d	1.40±0.02 ^d
G 4	2.17±0.01 ^b	2.34±0.03 ^b	2.63±0.06 ^b
G5	1.15±0.01 ^d	1.61±0.08 ^d	2.30±0.07 ^c
G 6	1.62±0.04 ^d	2.06±0.07 ^c	2.43±0.02 ^c

Means within the same column bearing different superscripts are significant at (p<0.05).

Table (5): The effect of Sulphatrim and Amoxi-vet on female estrogen (ng/mL) and progesterone (pg/mL) of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. (M±S.E) (n=5).

Fish Grouping	1 st day		7 th day		14 th day	
	Estrogen	Progesterone	Estrogen	Progesterone	Estrogen	Progesterone
G 1	2.22±0.07 ^a	2.18±0.04 ^a	2.42±0.07 ^a	2.32±0.03 ^a	2.43±0.07 ^a	2.34±0.02 ^{ab}
G 2	0.09±0.002 ^f	1.10±0.02 ^c	0.09±0.003 ^f	1.71±0.04 ^c	0.10±0.002 ^f	2.05±0.02 ^c
G 3	1.97±0.03 ^d	2.04±0.03 ^b	2.17±0.02 ^b	2.23±0.03 ^b	2.24±0.06 ^d	2.25±0.03 ^b
G 4	0.59±0.05 ^e	1.50±0.03 ^d	0.46±0.06 ^e	1.86±0.02 ^d	0.36±0.04 ^e	2.05±0.03 ^c
G5	1.05±0.02 ^c	1.67±0.02 ^b	1.76±0.06 ^c	2.12±0.03 ^c	2.04±0.05 ^c	2.26±0.02 ^d
G 6	0.89±0.04 ^d	1.34±0.02 ^e	1.55±0.04 ^d	1.79±0.03 ^{de}	1.90±0.02 ^d	2.12±0.01 ^c

Means within the same column bearing different superscripts are significant at (p<0.05).

Table (6): The effect of Sulphatrim and Amoxi-vet on serum AST (U/ml) and ALT(U/ml) of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. (M±S.E) (n=5).

Group	Days post-treatment											
	1 st day				7 th day				14 th day			
	AST		ALT		AST		ALT		AST		ALT	
	M	F	M	F	M	F	M	F	M	F	M	F
G 1	48.80± 1.77 ^d	51.80± 2.59 ^d	18.00± 1.30 ^c	18.60± 1.16 ^c	46.80± 1.06 ^c	52.20± 1.78 ^f	19.00± 1.09 ^c	19.80± 1.23 ^c	52.20± 0.73 ^{bd}	51.80± 1.39 ^{bc}	19.00± 0.44 ^c	18.80± 1.20 ^c
G 2	66.40± 2.22 ^a	69.20± 1.96 ^a	29.60± 1.43 ^a	32.20± 1.48 ^a	65.80± 0.58 ^a	65.00± 1.48 ^a	31.80± 0.80 ^a	33.80± 0.56	61.20± 1.35 ^a	60.00± 1.18 ^a	24.60± 0.97 ^a	25.20± 0.73 ^a
G 3	58.20 ± 2.26 ^b	62.80± 1.67 ^{ab}	27.40± 0.97 ^{ab}	26.00± 1.22 ^b	56.20± 1.35 ^b	59.00± 1.50 ^{ab}	20.00± 1.04 ^c	22.60± 1.13 ^d	56.00± 1.18 ^{bd}	54.20± 2.98 ^b	23.80± 1.01 ^c	22.40± 0.75 ^c
G 4	56.60± 1.53 ^{bc}	60.20± 0.53 ^{bc}	25.80± 0.73 ^b	27.80± 1.46 ^b	55.00± 0.83 ^b	57.40± 0.87 ^{bc}	22.60± 0.40 ^b	24.32± 2.46 ^c	55.60± 0.74 ^{bd}	54.80± 0.37 ^b	22.20± 2.01 ^{ab}	20.80± 0.58 ^{bc}
G 5	58.00± 0.89 ^{bc}	54.40± 1.14 ^{bd}	25.80± 1.01 ^b	29.20± 0.83 ^b	53.00± 1.54 ^b	52.60± 1.77 ^c	24.40± 0.67 ^b	25.40± 0.54 ^{bc}	48.00± 1.37 ^{cd}	50.00± 0.63 ^c	20.45± 0.74 ^{bc}	20.05± 1.09 ^{bc}
G 6	51.60± 1.40 ^{cd}	57.00± 1.30 ^{bcd}	26.40± 0.74 ^{ab}	26.30± 0.18 ^b	48.20± 1.56 ^c	50.20± 0.80 ^c	24.00± 0.54 ^b	26.20± 0.83 ^b	49.60± 1.43 ^{bc}	47.60± 1.12 ^c	20.36± 0.97 ^{bc}	20.60± 1.43 ^{bc}

Means within the same column bearing different superscripts are significant at (p<0.05).

Table (7): The effect of Sulptrim and Amoxi-vet on serum Urea (U/ml) and Creatinine (U/ml) of clinically healthy and experimentally infected *Oreochromis niloticus* with *A. hydrophila*. (M±S.E) (n=5).

Group	Days post-treatment											
	1 st day				7 th day				14 th day			
	Urea		Creatinine		Urea		Creatinine		Urea		Creatinine	
	M	F	M	F	M	F	M	F	M	F	M	F
G 1	0.23 ± 0.01 ^c	0.26 ± 0.01 ^c	0.51 ± 0.01 ^c	0.59 ± 0.01 ^c	0.25 ± 0.01 ^d	0.25 ± 0.01 ^c	0.54 ± 0.01 ^c	0.58 ± 0.02 ^b	0.25 ± 0.01 ^c	0.25 ± 0.01 ^c	0.51 ± 0.01 ^d	0.58 ± 0.02 ^b
G 2	0.59 ± 0.01 ^a	0.63 ± 0.02 ^b	0.71 ± 0.01 ^a	0.74 ± 0.01 ⁿ	0.59 ± 0.01 ^a	0.59 ± 0.01 ^b	0.71 ± 0.01 ^a	0.76 ± 0.01 ^a	0.62 ± 0.02 ^a	0.75 ± 0.02 ^a	0.74 ± 0.01 ^a	0.73 ± 0.01 ^c
G 3	0.34 ± 0.01 ^c	0.63 ± 0.01 ^c	0.59 ± 0.01 ^c	0.63 ± 0.01 ^b	0.33 ± 0.01 ^c	0.35 ± 0.01 ^b	0.62 ± 0.02 ^b	0.63 ± 0.01 ^b	0.29 ± 0.01 ^c	0.29 ± 0.01 ^c	0.56 ± 0.01 ^c	0.59 ± 0.01 ^b
G 4	0.29 ± 0.01 ^d	0.28 ± 0.01 ^c	0.55 ± 0.01 ^d	0.57 ± 0.01 ^c	0.28 ± 0.01 ^d	0.25 ± 0.01 ^c	0.57 ± 0.01 ^c	0.58 ± 0.01 ^b	0.28 ± 0.01 ^c	0.28 ± 0.01 ^c	0.54 ± 0.01 ^c	0.53 ± 0.01 ^{bc}
G 5	0.44 ± 0.01 ^b	0.47 ± 0.01 ^b	0.64 ± 0.01 ^b	0.64 ± 0.01 ^b	0.42 ± 0.02 ^b	0.38 ± 0.01 ^b	0.64 ± 0.01 ^b	0.70 ± 0.03 ^a	0.31 ± 0.01 ^b	0.33 ± 0.01 ^{bc}	0.52 ± 0.01 ^c	0.52 ± 0.02 ^{bc}
G 6	0.41 ± 0.02 ^b	0.36 ± 0.01 ^c	0.62 ± 0.01 ^{bc}	0.64 ± 0.01 ^b	0.36 ± 0.03 ^c	0.37 ± 0.01 ^b	0.69 ± 0.02 ^a	0.60 ± 0.03 ^b	0.34 ± 0.01 ^b	0.36 ± 0.01 ^b	0.65 ± 0.01 ^b	0.58 ± 0.01 ^b

Means within the same column bearing different superscripts are significant at (p<0.05).

REFERENCES

- Abu-Hegab, S.; Mohamed, A.; Marie, S. and Kandil, A. (1992)** : Toxicity of environmental pollutant on the aminotransferases activities of grass carp, *Ctenopharyngodonidella*. Bull. Zool. Soc., Egypt, 40; 19-33.
- Bauer, A. W.; Kibry, W. M. M.; Sherris, J. C. and Turck, M. (1966)** : Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45; 493-496.
- Bayazit et al., (2004)** : Cytotoxic effects of some animal and vegetable extracts and some chemicals on liver and colon carcinoma and myosarcoma. Saudi Med J. 25(2):156-63.
- Becton, D. (1977)** : Becton Dickinson Vacutainer Systems. laboratory procedures using the Unopette Brand System.
- Berglund, F. (1976)** : Effect of trimethoprim-sulfamethoxazole in the renal excretion of creatinine in man. J. Urol., 114, 802-808.
- Castilas, E.; Myer, M. and Ames, W. (1983)** : Relationship of serum chemistry values to liver and kidney histopathology in English sole (*Parpphrys vetulus*) after acute exposure to carbon tetrachloride. Aquatic Toxicology, 3 ; 61-78.
- Ceylanae, E.; Berktasb, M.; Korkocab, H.; Kelesa, I. and Bozkurtb, H. (2003)** : Prevalence and Antibiotic Sensitivity of Motile *Aeromonas* in Dogs. ACTA VET. 72: 607-612.
- Dijkmans, B. A.; Van Hoof, J. P.; De Wolff, F. A. and Muntje, H. (1982)** : The Effect of Co-Trimoxazole on serum creatinine. Br. J. Clin. Pharmac. 12, 701-703.
- Doumas, B. T. (1975)** : Colorimetric determination of total proteins. Clinical., Chem. 21:1159-1166.
- Doumas, B. T. and Biggs, H. G. (1972)** : Determination of serum globulin . In Standard Method of Clinical Chemistry. Vol. 7. Edited by G.R.Cooper. New York, Academic press.
- Doumas, B. T.; Baysa, D. D., Carler, R. J., Peler, T. and Schaffer, R. (1981)** : Determination of serum albumin. Clinical ., Chem., 27:1642.
- Foo, J. T. and Lam, T. J. (1999)** : Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the tilapia, *Oreochromis mossambicus*. Aquaculture, 115 . 145-158.
- Gracia, T.; Hilscherova, K.; Jones, P. D.; Newsted, J. L. and Higley, E. B. (2007)** : Modulation of steroidogenic gene expression and hormone production of H295R cells by pharmaceuticals and other environmentally active compounds. Toxicol. Appl. Pharmacol. 225 , 142-153.
- Henry, R. J. (1974)** : Colorimetric Determination of Creatinine Chemistry, Principles and Techniques. 2nd Ed. Harper and Row. P. 525.
- Hood, V. L.; Hall, B. M.; Horvath, J. S.; Jones, B.; Johnd, J. R. and Tiller, D. J. (1975)** : Trimethoprim /sulfamethoxazole and renal function in transplant patients. Austr. N. Z. J. Med., 6, 86.
- Iain, D.; Neil, M. R.; Michelle, G. and Cyril, C. (2007)** : The advantages of the use of discs containing single agents in disc diffusion testing of the susceptibility of *Aeromonas salmonicida* to potentiated sulphonamides. Aquaculture, 272 .118-125.
- Inglis, V.; Soliman, M. K. and Higuera, I. (1992)** : Amoxycillin in control of frunculosis in Atlantic salmon. Veterinary Record ; 130:45-48.
- Iunes, W. T. (1966)** : Exotic Aquarium Fishes. 4th Ed. Aquar.Inc. Gresy, P. 530-533.

Jindal, N.; Garg, S. R. and Kumar, A. (1999): Comparison of *Aeromonas* spp. isolated from human, livestock and poultry faeces, *Isr J Vet Med* 48: 80-83.

Lee, H. A. and Hill, L. F. (1968): The use of ampicillin in renal disease. *Br. J. clin. Pract.*, 22, 354-357.

Lockhart, W. L. and Mentner, D. A. (1984): Fish Serum Chemistry as a pathology tool. In *Contaminant Effects of fishers*. 16 : 73-86.

London, R. D. (1967): Hematuria associated with methicillin therapy. *J. Pediat.*, 70, 285-286.

Mark E. P. Howa, David Perrett and Jack Kay (1997): Optimisation of a simultaneous separation of sulphonamides, dihydrofolate reductase inhibitors and B-lactam antibiotics by capillary electrophoresis. *Journal of Chromatography A*, 768 . 97-104 .

Miller, S. D. and Richards R. H. (1993): Resistance of *Aeromonas salmonicida* to amoxicillin. *Journal of Fish Diseases* Volume 16, (4), 389-395.

Mottelb, E. (1972): A study on the changes of blood in buffalo calves suffering from enteritis due to different causative agents. Thesis Fac. Vet. Med. Assuit Univ. Assiut, Egypt.

NCCLS, National Committee for Clinical Laboratory Standards (1994): Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals, proposes standard. Publication M 31-p NCCAS Document , 14; 20.

Park, S. and Choi, K. (2008): Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. *Ecotoxicology*.17, 526-538.

Patton, C. J. and Crouch, S. R. (1977):

Enzymatic determination of urea. *Anal. Chem.*49:466-469

Plumb, J. A. (1999): Tilapia bacterial diseases. In: Plumb, J.A. (Ed.). *Health Maintenance and Principal Microbial Diseases Cultured Fishes*. Iowa State Univ. Press, AMES, pp. 297-305.

Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R.(1994): *Clinical Veterinary Microbiology*. Wolfe, London, England. ISBN 072341-7113.

Racicot, J. G.; Gaunt, M. and Leray, C. (1975): Blood and liver enzymes in rainbow trout (*Salmogairdneri Rich*)with emphasis of *Aeromonas* infections. *J. Fish Biol* .vol. 7. P. 825-835.

Riedmuller, S. (1966): Electrophoretic blood protein research in healthy and infected carp. *Bull Off. Int . Epiz.* , 65(6):745-750 .

Reitman, S. and Frankel, S. (1957): Colourimetric Determination of glutamic oxaloacetic and glutamic pyruvic transaminase .*Am. J. Clin. Path.*, 28; 56 .

Rurangwa, E.; Kime, D. E.; Ollevier, F. and Nash, J. P. (2004): The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 234: 1 - 28.

Sanjay, B. and Anil, D. (2006): Acute liver failure. *Current Paediatrics* 16, P. 36-40.

Shoemaker, C. and Klestus, P. (1997): Streptococcal disease problems and control a review. In: Fitzsimmons, K. (Ed.). *Tilapia Aquaculture 2 Northeast Regional Agricultural Engineering Service*. Ithaca, NY, pp. 671-682.

Snedecor, G . W. and Cochoran, N. G. (1967): *Statistical Methods*. (6th ed) .The Iowa State University Press. Ames.

Tietz (1987): Turbidimetric measurement

of lipase activity-problems and some solutions. *Clin Chem.* 1987 Sep;33(9):1624-9.

Verma, S. R.; Rami, S. and Dalela, R. C. (1981): Isolated and combined effect of pesticides on serum transaminases in *Mystus Vitatus*. *Toxicol.Lett.* 9:67-71.

World Health Organization (1992): Labor-

atory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 3rd ed. Cambridge University Press. New York.

Wright, P. A. and Land, M. D. (1998) : Urea production and transport in teleost fishes. *Comp. Biochem. Physiol.* 119: 47-54.

الملخص العربي

مدى كفاءة كلاً من عقار السلفا تريم والأموكسى فيت ضد العدوى بميكروب الأيرومونات هيدروفيليا في سمك البلطي النيلي

محمد جبر مجدي عامر ريهام أحمد الشافعي

قسم الأدوية - كلية الطب البيطري - جامعة المنصورة

تعتبر الأسماك أهم وأرخص مصادر البروتين الحيواني في غذاء الإنسان خاصة في الدول محدودة الدخل. ومع تزايد عدد مزارع الأسماك في الوقت الحالي أدى إلى انتشار الكثير من مسببات العدوى البكتيرية بين تلك المزارع ومن بين تلك المسببات الإصابة بميكروب الأيرومونات هيدروفيليا والذي يؤدي إلى فقد كبير في إنتاج هذه الصناعة. لذا أجريت هذه الدراسة لتقييم استخدام كلا من عقار السلفا والأموكسيسيلين ضد ميكروب الأيرومونات هيدروفيليا وكذا تأثيره علي صورة الحيوانات المنوية في سمك البلطي النيلي. ولقد استخدم في هذه الدراسة عدد 144 سمكة قسمت الي ست مجموعات علي النحو التالي: المجموعة الأولى (ضابط سلبي) والمجموعة الثانية (معداه - ضابط إيجابي) والمجموعة الثالثة (غير معداه ومعالجه بالسلفا بجرعة 25 مجم/كجم وزن حي) والمجموعة الرابعة (غير معداه ومعالجه بالأموكسيسيلين بجرعة 80 مجم/كجم وزن حي) والمجموعة الخامسة (معداه ومعالجه بالسلفا 25 بجرعة مجم/كجم وزن حي) والمجموعة السادسة (معداه ومعالجه بالأموكسيسيلين 80 بجرعة مجم/كجم وزن حي) ثم قسمت كل مجموعته إلي مجموعتان فرعيتان (ذكر وأنثى) كل منها 15 سمكة. ولقد أعطي السمك المعدي بميكروب الأيرومونات هيدروفيليا عن طريق الحقن البريتوني.

تم تقييم القدرة العلاجية من خلال ملاحظه الأعراض المرضية ونسبة النفوق كما تم أخذ عينات الدم لفصل المصل وتقييم وظائف الكبد والكلي. كما تم أيضا دراسة تأثير كلاً من هذان العقاران علي صورة الحيوانات المنوية ونسبة الهرمونات الذكرية و الأنثوية في الأسماك المعالجة.

وقد أسفرت النتائج عن مدى فاعلية عقار السلفا في علاج ميكروب الأيرومونات هيدروفيليا في سمك البلطي النيلي بالمقارنة بالأموكسيسيلين وذلك من خلال تحسين الأعراض المرضية ونسبة النفوق كما أن المجموعات المعالجة بعقار السلفا لم تظهر تغير ملحوظ في وظائف الكبد و الكلي. هذا وسجلت النتائج نقصا في عدد الحيوانات المنوية في المجموعه المعالجة بعقار الأموكسيسيلين بينما لم يظهر تغيرات معنوية في عدد الحيوانات المنوية ونسبة هرمونات الذكر و الأنثى في مجموعات البلطي المعالج بالسلفا.