Vet.Med.J.,Giza. Vol.46, No.4 . A (1998): 555-567.

EFFECT OF MALACHITE GREEN ON THE EFFICACY AND PHARMACOKINETICS OF OXYTETRACYCLINE WITH SPECIAL REFERENCE TO THEIR BIOCHEMICAL AND HORMONAL CHANGES IN EXPERIMENTALLY INFECTED *CLARIAS LAZERA*.

By

K. ABO EL-SOOUD* AND H.I. ABASS**

Departments of Pharmacology* and Physiology**; Faculty of Veterinary Medicine; Cairo University; Giza 12211; Egypt.

SUMMARY:

The effect of malachite green on the efficacy, pharmacokinetics and tissue depletion of oxytetracycline was studied in *Clarias lazera* (catfish) experimentally infected with *Aeromonas hydrophila* . Infected fish were exposed to oxytetracycline (100 mg L^{-1}) as a bath with or without malachite green (2 mg L^{-1}) for 15 minutes daily for 7 consecutive days. The obtained results showed that malchite green augmented the efficacy of oxytetracycline by reducing the mortalities and enhanced its elimination from serum and tissues. The elimination half-life was ($T_{1/2el}$) of 11.748 ± 0.52 hours and mean residence time (**MRT**) was of 17.337 ± 0.88 hours. Residues of oxytetracycline were detected in muscles up to 15 days after the cessation of treatment when it was given alone. While malachite green enhanced the residue depletion of oxytetracycline from muscles, as it could not be detected up to 10 days in fish treated with both agents. The combination of oxytetracycline and malachite green increased the gonadal secretion of sex steroids (either testosterone or 17-β estradiol) via elevation of gonadotropin (LH) levels in both sexes. The liver and kidney functions and glucose utilization were also improved in experimentally infected fish treated with both drugs rather than the use of oxytetracycline alone allover the withdrawal periods.

INTRODUCTION

The intensive fish rearing (over-crowded) eventually increased the incidence of spreading infections from one aquarium to another especially **Motile Aeromonas Septicaemia (MAS)**, among the cultured fish in Egypt. *Aeromonas hydrophila* is the causative agent of MAS is being considered

as one of the serious pathogenic bacteria in warm-water aquaculture throughout the world (**Thune *et al.*, 1993**). Many protozoa infecting skin and gills of fish become parasitic in the presence of another disease conditions **(Hoffman , 1974)** . So, if the fish is infected with bacterial infection, the susceptibility for parasitic infection has been increased. Oxytetracycline is a commonly used antibiotic in commercial aquaculture of freshwater and marine fish species. It is given to farmed fish to minimize losses from many infectious diseases **(Bruno, 1989)** . Oxytetracycline proved to be effective against *Aeromonas hydrophila* in fish (**Krovacek *et al.*, 1989 and Sarma, 1990**). Also, administration of oxytracycline to mature females before spawning significantly reduced the prevalence of infection transmission to the eggs at the hatch stage and improved the hatching percent (**Brown *et al* ., 1990**) . Malachite green is being an effective topial ectoparasiticide and also antiprotozoal agent against the protozoan "PKX" causative agent of Proliferative Kidney Disease in fish **(CIifton- Hadley and Alderman, 1987)**. Most aquaculturists used the malachite green and formalin baths as prophylactic regimen for protozoan parasites in fish that kept in both steel tanks and earth ponds . This regimes increased the survival rate of fingerlings and fucundity of adult fish up to 2-years age **(Rintamaki-Kinnunen and Valtonen 1997)**. Therefore, it is essential to use antiprotozoal agents with the antibacterial drug when the infection occurred. No literature is available concerning the use of both groups of drugs together. Therefore, the aim of the present work was to study the effect of malachite green on the efficacy; pharmacokinetics and tissue depletion of oxytetracycline in *Aeromonas hydrophila* infected *Clarias lazera* (catfish) . Another goal was to study the effects of this combination on the liver and kidney functions and the levels of sexual and gonadotropic hormones on both sexes of Clarias fish .

MATERIALS AND METHODS

Drugs:

1- Oxytetracycline (Terramycin @) obtained as (30 mg ml^{-1}) bottle solution from **Pfizer Co., Egypt**.

2- Malachite green : was available in the form of crystals with metallic luster, highly soluble in water obtained from **(Sigma Chemicals Co., USA)**.

Fish:

A total number of 105 apparently healthy freshwater Nile catfish (*Clarias lazera*) o both sexes with an average body weight o 250 grams aging about 6 months wer obtained from a private fishery. Fish wer kept in glass aquaria supplied wit dechlorinated water and thermostaticall adjusted at 20±1°C. Fish were fed o commercial pelleted feed and acclimated f 14 days before experiments.

Bacteria :

Aeromonas hydrophila, obtained from (**Fish Diseases and Management Dept., Faculty of Vet. Med., Cairo University)** was used for experimental infection of catfish. The organism was subcultured in nutrient broth for 24 hours at 22°C and then adjusted to 0.5 McFarland barium sulphate standard solution (corresponding to $1x10^8$ cfu/ml) . The count was expressed in **cell forming count.**

Experimental design:

A total number of 105 clinically normal mature Nile catfish *Clarias lazera* of both sexes were divided into four groups **as follows:**

The first three groups of 30 fish each were experimentally infected intraperitoneally with 0.2 ml of 24 hours pure broth culture of ***Aeromonas hydrophila*** (total bacterial count $1x10^8$ cfu/ ml). The inoculated fish were observed for the development of clinical signs indicating Aeromonas infection 24 hours post bacterial inoculation and grouped as follows:

The **First group** was kept as *infected non-treated (control +ve) group .*

The **second group** was treated with oxytetracycline 100 mg L^{-1} as a bath for 15 minutes daily for 7 consecutive days.

The **third group** was treated with both oxytetracycline 100 mg L^{-1} and malachite green 2 mg L^{-1} for 15 minutes together in the same tank for 7 consecutive days.

The **fourth group** of 15 fish was kept as *non-infected non-treated (control -ve) group* . They were injected once intraperitoneally with 0.2 ml saline solution.

The treatment solutions were prepared 15 hours before use in order that the equilibrium could be established . After exposure to treatments, the fish were returned to their tanks until sampled.The percentages of mortalities in all groups were recorded daily and dead fish as well as recovered fish were bacteriologically examined.

Pharmacokinetic study:

Fish in the 2^{nd} and 3^{rd} group were used for pharmacokinetic study . After the first exposure to oxytetracycline alone or with malachite green, blood samples were collected from the caudal vein of 10 fish before and at 0.25, 0.5 , 1, 2, 4 , 6 , 8, 10 and 24 hours and once every day for 21 days post exposure to the treatment solutions. Serum samples were separated from clotted blood by centrifugation at 3000 rpm for 15 minutes and kept until analyzed. Serum protein binding of oxytetracycline was estimated *in vitro* according to **Lorian (1980).**

Tissue residue study:

After 7 days of treatment , three fish were killed from the 2nd and 3rd groups at different intervals (1,5,10,15 and 21 days) to determine oxytetracycline concentrations in muscles and its withdrawal time. Muscles were homogenized with 90% methanol containing 0.5% EDTA to extract oxytetracycline from the tissues then they were centrifuged. The supernatant were evaporated and diluted with 0.5ml of distilled water to be analyzed (**Ueno and Aoki, 1995**).

Concentrations of oxytetracycline in sera and muscle extracts were assayed by microbiological method using ***Bacillus subtilis*** as standard organism (**Kusser and Newman, 1990**).

Hormonal and biochemical study:

The level of sexual hormones on both sexes and liver and kidney functions were studied in all four groups. Blood samples were collected from the caudal vein of 7 fish from each sex via the puncture of the caudal peduncle at a fixed time (9.00 a.m.) to avoid the diurnal fluctuation in the enzymatic and hormonal profiles at the 1st, 7th and the 15th day after the last exposure to the treatment solutions. Serum samples were separated from clotted blood by centrifugation at 3000 rpm for 15 minutes and kept at -20°C until analyzed.

Hormonal assay : Radio-immunoassay (RIA kits) technique was used for estimating the level of sexual hormones. Serum testosterone level was determined according to the method of **Jaffe and Behrman (1974).** Serum 17-β estradiol level was determined according to the method of **Xing *et al.*, (1983)** . Serum luteininzing hormone was determined according to the method of **Kawauchi , *et al.*, (1989)**.

Liver function tests: The serum level of transaminases **(AST & ALT)** was determined colorimetrically according to the method of **Reitman and Frankel (1957)**.

Kidney function tests : The serum level of urea was determined colorimetrically according to the method of **Fawcett and Scott (1960)** and serum creatinine was determined according to the method of **Husdan and Rapoport (1968)**.

Serum glucose was determined according to the method of **Trinder (1969)**.

Pharmacokinetic analysis:

A computerized curve-stripping program **(Rstrip, Micromath Scientific Software, Salt Lake City, UT, and USA)** was used for data analysis for each fish after exposure to oxytetracycline. Each individual curve of oxytetracycline vs time was analyzed to determine peak concentration (C_{max}), time to peak concentration (T_{max}), elimination

half-life ($T_{1/2el}$), area under the curve (AUC) from zero to infinity by the trapezoidal integral and mean residence time (MRT) in serum. All data were tabulated as mean ± SEM.

Statistical analysis :

The obtained results were statistically analyzed using Student's t test.

RESULTS

Regarding bacterial infection , clinical signs of catfish experimentally infected with *Aeromonas hydrophila* manifested in most fish by dermal ulceration, focal haemorrhage and inflammation . Skin and muscle erosions were also seen especially over the abdominal area. As shown in **(Table 1)** mortalities were markedly reduced in the group treated with oxytetracycline and malachite green followed by that treated with oxytetracycline alone. Bacteriological examination of the dead fish revealed the presence of *Aeromonas hydrophila*, whereas the bacteria were not reisolated in any fish received oxytetracycline.

Concerning the pharmacokinetic data , the results showed that oxytetracycline is fairly absorbed through the bath: gill route (the heavily vascularized gill is here assumed to be the principle route of oxytetracycline uptake from solution) . The mean peak concentration of oxytetracycline (C_{max}) in catfish after exposure to 100 mg L^{-1} for 15 minutes was 0.425 ± 0.012 ug ml^{-1} at (T_{max}) 1.160 ± 0.11 hours**(Fig.1)** Malachite green did not influence oxytetracycline absorption when both agents mixed together.The elimination half-life was ($T_{1/2el}$) of 11.748 ± 0.52 hours and mean residence time (MRT) was 17.337 ± 0.88 hours **(Table 2)**. Malachite green accelerates the elimination of oxytetracycline by decreasing the half-life of elimination ($T_{1/2el}$) to 10.171 ± 0.43 hours and mean residence time (MRT) to 15.031 ± 0.56 hours . The protein binding of oxytetracycline to the serum of catfish was 19.612 ± 0.751% indicating that the drug has a relatively low extent of binding to the serum proteins. Oxytetracycline concentrations in the muscles were detected up to 15 days after the cessation of treatments.

The mean oxytetracycline concentrations in sera and muscles of catfish after exposure to 100 mg L^{-1} for 15 minutes for 7 consecutive days are incorporated in **Table 3**. Malachite green enhances the depletion of oxytetracycline from tissues, as it could not be detected up to 10 days in the group treated with both chemotherapeutic agents. The results in **Table 4 & 5** revealed that treatment of infected fish with oxytetracycline and malachite green together improved the glucose absorption and gonadal functions (testes and ovaries) via the elevation of sera levels of both testosterone and 17- β

Table 1 : The effect of oxytetracycline with or without malachite green on mortalities in *Aeromonas hydrophila* infected *Clarias lazera* (catfish).

Group	Mortality percent
1- Non-infected, non-treated.	0%
2- Infected, treated with oxytetracycline only.	18%
3- Infected, treated with oxytetracycline and malachite green.	7%
4- Infected, non-treated.	50%

Table 2 : Pharmacokinetic parameters of oxytetracycline in *Aeromonas hydrophila* infected *Clarias lazera* (catfish) after exposure to 100 mL^{-1} for 15 minutes. (n = 10).

Parameter	Unit	Fish treated with OXY.	Fish treated with OXY + MG.
A	ug ml^{-1}	0.425 ± 0.010	0.480 ± 0.015*
K_{ab}	h^{-1}	3.60 ± 0.52	2.90 ± 0.37
$T_{1/2ab}$	min	11.563 ± 1.35	14.326 ± 1.45
B	ug ml^{-1}	0.419 ± 0.006	0.470 ± 0.009**
K_{el}	h^{-1}	0.059 ± 0.003	0.068 ± 0.004
$K_{1/2el}$	h	11.748 ± 0.520	10.171 ± 0.435*
C_{max}	ug ml^{-1}	0.425 ± 0.012	0.445 ± 0.014
T_{max}	h	1.160 ± 0.11	1.132 ± 0.12
AUC	ug/ml/min	419.118 ± 11.32	404.981 ± 9.51
MRT	h	17.337 ± 0.88	15.031 ± 0.56*

** Significant at P < 0.05 ** Significant at P < 0.005.

Table 3 : **Oxytetracycline concentrations (ug ml^{-1} or ug g^{-1}) in serum and muscles after exposure as a bath for 15 minutes to 100 mg L^{-1} for 7 consecutive days in *Aeromonas hydrophila* infected *Clarias lazera* (catfish). (n = 3).**

Withdrawal days	FISH TREATED WITH OXY. ALONE		FISH TREATED WITH OXY + MG	
	Serum	Muscle	Serum	Muscle
1	1.120 ± 0.02	0.962 ± 0.019	1.305 ± 0.30	0.807 ± 0.021
5	0.375 ± 0.027	0.612 ± 0.011	0.245 ± 0.020	0.480 ± 0.022
10	0.062 ± 0.031	0.314 ± 0.017	0.041 ± 0.012	0.250 ± 0.010
15	ND	0.087 ± 0.010	ND	ND
21	ND	ND	ND	ND

ND : Not detected

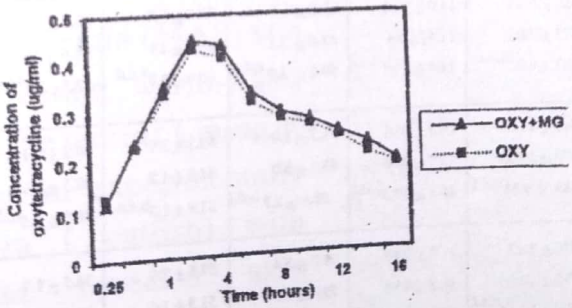

Figure 1: Semilogarithmic graph depicting the time-concentration course of oxytetracycline in serum of catfish after exposure for 15 minutes to 100 mg/L with or without malachite green.

Table 4 : Effect of oxytetracycline with or without malachite green on some hormonal levels and biochemical parameters in experimentally infected male (♂) *Clarias lazera* (n = 7).

Parameter / Group	Days after the last exposure	Serum testosterone (ng ml $^{-1}$)	Serum LH (mIU ml $^{-1}$)	Serum glucose (mg%)	Serum AST (U/L)	Serum ALT (U/L)	Serum creatinine (mg/dl)	Serum urea (mg/dl)
Non-infected-non-treated control (-ve)	1	0.58 ± 0.02	38.6 ± 00.4	120 ± 3.68	36.4± 1.4	20.3 ± 1.1	0.52 ±0.03	23.6 ± 1.4
	7	0.60 ± 0.03	38.4 ± 00.4	118 ± 3.22	37.0± 1.1	20.0 ± 1.3	0.54 ± 0.02	23.5 ± 1.7
	15	0.59 ± 0.03 ^{a,b,c}	38.9 ± 00.5 ^{a,b,c}	122 ± 3.8 ^{a,b,c}	35.7 ± 1.4 ^{a,b,c}	20.6 ± 1.0 ^{a,b,c}	0.53 ± 0.02 ^{a}	24.8 ± 1.5 ^{a,b,c}
Infected, non-treated control (+ve)	1	0.16 ± 0.02	13.3 ± 00.5	60.3 ± 3.1	75.6 ± 2.2	56.3 ± 4.0	0.85 ± 0.03	60.8 ± 2.6
	7	0.18 ± 0.02	13.7± 00.1	63.5 ± 2.8	73.8 ± 2.8	58.0 ± 3.0	0.80 ± 0.03	58.2 ± 4.1
	15	0.19 ± 0.02 ^{a,d,e}	14.0 ± 00.3 ^{a,d,e}	65.4 ± 3.4 ^{a,d,e}	71.4 ± 3.8 ^{a,d,e}	58.6 ± 3.8 ^{a,d,e}	0.77 ± 0.03 ^{a,b,c}	57.0 ± 3.0 ^{a,d,e}
Infected and Treated with Oxytetracycline	1	0.28 ± 0.03	22.2 ± 00.4	77.8 ± 3.0	57.0 ± 2.0	37.6 ± 2.1	0.60 ± 0.03	50.1 ± 1.9
	7	0.32 ± 0.01	26.0 ± 00.3	87.2 ± 2.7	52.8 ± 3.8	35.4 ± 1.1	0.57 ± 0.03	48.2 ± 2.1
	15	0.35 ± 0.03 ^{b,d,f}	30.7 ± 00.4 ^{b,d,f}	90.6 ± 2.0 ^{b,d,f}	50.4 ± 2.1 ^{b,d,f}	33.8 ± 0.8 ^{b,d,f}	0.55 ± 0.04 ^{b}	46.3 ± 1.6 ^{b,d,f}
Infected and Treated with oxytetracycline + malachite green	1	0.30 ± 0.03	24.6 ± 00.3	78.3 ± 4.0	55.2 ± 3.6	34.5 ± 1.9	0.60 ± 0.06	44.0 ± 1.9
	7	0.37 ± 0.02	37.7 ± 00.4	88.7 ± 3.1	50.4 ± 3.8	30.2 ± 1.6	0.58 ± 0.04	40.2 ± 1.7
	15	0.45± 0.01 ^{c,e,f}	33.7± 00.5 ^{c,e,f}	100 ± 2.2 ^{c,e,f}	42.3 ± 1.0 ^{c,e,f}	25.8 ± 1.1 ^{c,e,f}	0.54 ±0.04 ^{c}	36.8 ± 1.8 ^{c,e,f}

Mean ± S.E, mean values having the same letter (s) in the same column are significantly different from each other at P < 0.01.

Table 5 : Effect of oxytetracycline with or without malachite green on some hormonal levels and biochemical parameters in experimentally infected female (♀) *Clarias lazera* (n = 7).

Parameter / Group	Days after the last exposure	Serum 17 β- estradiol (pg ml $^{-1}$)	Serum LH (mIU ml $^{-1}$)	Serum glucose (mg%)	Serum AST (U/L)	Serum ALT (U/L)	Serum creatinine (mg/dl)	Serum urea (mg/dl)
Non-infected-non-treated control (-ve)	1	1089 ± 12.40	24.8 ± 00.3	88.4 ± 4.00	42.2 ± 1.5	26.2 ± 1.2	0.52 ±0.03	26.1± 1.3
	7	1092 ± 13.76	25.0 ± 00.4	89.6 ± 3.90	40.7± 1.6	26.6 ± 1.3	0.57 ± 0.04	27.2 ± 1.2
	15	1080 ±14.8 ^{a,b,c}	24.5 ± 00.4 ^{a,b,c}	92.8 ± 3.0 ^{a,b,c}	42.7 ± 1.0 ^{a,b}	27.4 ± 0.8 ^{a,b,c}	0.52 ± 0.05 ^{a}	26.2 ± 1.5 ^{a,b,c}
Infected, non-treated control (+ve)	1	350 ± 5.73	11.0 ± 00.4	43.0 ± 4.0	90.6 ± 5.6	60.8 ± 4.0	0.89 ± 0.06	64.4 ± 4.0
	7	372 ± 7.85	11.4± 00.4	47.4 ± 3.1	82.2 ± 4.9	55.6 ± 3.1	0.83 ± 0.04	61.3± 3.8
	15	382 ± 6.00 ^{a,d,e}	11.6 ± 00.5 ^{a,d,e}	50.2 ± 2.9 ^{a,d,e}	80.6 ± 3.7 ^{a,c,d}	52.3± 3.0 ^{a,d,e}	0.80 ± 0.04 ^{a,b,c}	58.7 ± 3.1 ^{a,d,e}
Infected and Treated with Oxytetracycline	1	545 ± 11.34	14.3 ± 00.4	58.7 ± 3.0	62.3 ± 2.0	40.2 ± 2.1	0.62 ± 0.05	47.2 ± 3.1
	7	576 ± 10.28	15.0 ± 00.3	62.3 ± 3.0	54.0 ± 1.2	38.1 ± 2.6	0.60 ± 0.04	45.3 ± 2.1
	15	589 ± 9.88 ^{b,d,f}	15.6 ± 00.3 ^{b,d,f}	65.4 ± 2.7 ^{b,d,f}	51.8 ± 1.3 ^{b,c,e}	38.0 ± 1.0 ^{b,d,f}	0.57 ± 0.04 ^{b}	40.4 ± 1.2 ^{b,d,f}
Infected and Treated with oxytetracycline + malachite green	1	750 ± 8.23	17.8 ± 00.3	69.9 ± 3.4	57.8 ± 2.0	36.5 ± 1.2	0.60 ± 0.03	40.2 ± 1.9
	7	772 ± 10.20	18.2 ± 00.4	72.4 ± 2.9	51.3 ± 1.2	34.1 ± 1.1	0.58± 0.02	38.6 ± 2.1
	15	800 ± 12.64 ^{c,e,f}	19.8± 00.5 ^{c,e,f}	78.8 ± 2.2 ^{c,e,f}	44.6 ± 1.5 ^{d,e}	32.0 ± 1.0 ^{c,e,f}	0.56 ±0.03 ^{c}	30.7 ±1.1 ^{c,e,f}

Mean ± S.E, mean values having the same letter (s) in the same column are significantly different from each other at P < 0.01.

estradiol in male and female Clarias fish, respectively . Moreover, serum luteinzing homone levels in both male and femal Nile catfish were significantly higher in treated groups as compared to infected non treated group. The results of the experimental investigation **(Table 4 & 5)** showed that exposure of infected catfish to oxytetracycline and/ or malachite green in a therapeutic dose induced a significant decrease in serum transaminases (AST & ALT) levels as compared to infected- non treated control. Oxytetracycline application either alone or with malachite green had a significant reduction effect on serum creatinine and urea levels as compared to infected - non treated fish ($P<0.01$). Contrary to blood urea, serum creatinine levels in treated groups were not significantly different to those of non infected- non treated (control - ve)values ($P<0.01$).

DISCUSSION

Motile Aeromonas Septicaemia (MAS) is a major fish disease affecting warm-water aquaculture throughout the world (**Leung *et al.*, 1996**). Examination of experimentally infected *Clarias lazera* (catfish) with *Aeromonas hydrophila* revealed skin ulceration, focal haemorrhage and inflammation. These findings resemble those of **Huizinga *et al.*, 1979** and **Leung *et al.*, 1996**. In the present study most of clinical signs of infection were disappeared and mortalities were significantly reduced in groups treated with oxytetracycline. These results are consistent with those of **Bayer and Daniel (1987)** who found that the mortality was 15% for group treated with oxytetracycline. Moreover, **Krovacek *et al.*, 1989 and Sarma, 1990** found that *Aeromonas hydrophila* was highly sensitive to oxytetracycline. In general, when two drugs are administered simultaneously they may interact to augment or diminish the expected response or to cause unanticipated toxicity. In this study malachite green augments the efficacy of oxytetracycline against *Aeromonas hydrophila* infection by reducing the mortality precent more than oxytetracycline alone. The serum and tissue concentrations of oxytetracycline were higher than the minimum inhibitory concentration (MIC) against Aeromonas infection (0.25 ug ml^{-1}) for at least five days after exposure to the drug. **Barnes et al., (1995)** found that the MIC of oxytetracycline was increased in marine -water due to high magnesium content. The disposition of oxytetracycline in catfish after exposure to 100 mg L^{-1} for 15 minutes (as a bath) is similar to that reported by **Uno *et al.*, (1992)**. The calculated values for (C_{max}) were 0.425 ug ml^{-1} and 0.445 ug ml^{-1} were obtained at (T_{max}) 1.160 and 1.132 hrs. in catfish treated with oxytetracycline and oxytetracycline with malachite green, respectively. The elimination half-life ($T_{1/2el}$) of oxytetracycline was 11.748

hours, shorter than those reported by **Uno *et al.*, (1992)** in normal rainbow trout, amago salmon and yellow tail (23, 16 and 28 hrs.), respectively. This is may be due to presence of infection in our study. It was found that malachite green enhances the elimination of oxytetracycline by decreasing the elimination half - life ($T_{1/2el}$) to 10.171 hours and mean residence time (MRT) to 15. 031 hours. The capacity of oxytetracycline to bind with serum proteins of catfish was 19.612%. This relatively low extent of binding is consistent with the extensive distribution to tissues. Oxytetracycline residues in the muscles of catfish could be detected up to 15 days after the cessation of treatment . This finding is similar to those obtained by **Bayer and Daniel (1987)** and **Xu and Rogers (1994)**. They found that oxytetracycline could be detected in muscles from 12 to 18 days after stopping of medication . It was observed also that malachite green enhances the depletion of oxytetracycline from tissues, as it could not be detected up to 10 days in the group treated with both chemotherapeutic agents.

Treatment of infected fish with oxytetracycline and malachite green improved the glucose absorption and gonadal functions (testes and ovaries) via the elevation of serum levels of both testosterone and 17-β estradiol, respectively. Moreover, serum luteinzing hormone levels in both male and female Nile catfish were significantly higher in treated groups as compared to infected non treated group. **Brown et al., 1990** and **Kudo and Yazawa (1997)** found that administration of oxytetracycline to mature broodstock females before spawning significantly reduced the prevalence of infection transmission to the eggs at the hatch stage and improved the hatching percent. They suggested that the vitelline or fertilization envelopes may have the ability to protect egg itself or embryo, respectively by trapping drug. Also **Rintamaki-Kinnunen and Valtonen (1997)** found that malachite green bath increased the hatching and survival rates of the fertilized eggs. So, the use of malachite green with oxytetrarcycline is solely safe in broodstock fisheries as they may improve the fecundity and hatching percent. The blood glucose level of infected non-treated (control +ve) group was significantly decreased than those of treated and control -ve groups, this may be due to anorexia during the infection **(Fletcher , 1984)**. Exposure of fish to drugs might improved the glucose absorption and utilization in treated fish . The results of the experimental investigation showed that exposure to oxytetracycline in therapeutic dose either alone or with malachite green induced a significant increase in serum transaminases (AST & ALT) of infected catfish as compared to non infected Clarias fish(control-ve). But serum transaminases levels are significantly lowered than those respective values of infected non-treated fish

at 15 days from stopping of medications. These results are in agreement with **Racicot *et al.*, (1975)** and **Sarma (1990)**, who found that *Aeromonas hydrophila* infection caused continuous destructive changes in the hepatic cells that led to elevation in serum transaminases. In this respect, **Breen *et al.* (1975)** suggested that tetracyclines caused hepatic dysfunction and azotaemia. So, malachite green improved the effect of oxytetracycline on liver function.

Oxytetracycline application either alone or with malachite green had a significant reduction effect on creatinine and urea levels as compared to infected non treated fish. Serum creatinine levels in treated groups of both sexes were not significantly different to those of non infected non-treated group (control-ve) values. The explanation of that may be attributed to the fact that creatinine is readily eliminated than urea. So that an increase in creatinine level may not be seen early as that in urea . So, advanced renal impairment by the effect of sever Aeromonas infection is expected without drug administration **(Li *et al.*, 1993)**.

REFERENCES

Barnes, A.C.; Hastings, T.S.& Amyes, S.G.B. (1995) : Aquaculture antibacterials are antagonized by seawater cations. Journal of Fish Diseases, 18 , 463 - 465.

Bayer, R.C.& Daniel, P.C. (1987) : Safety and efficacy of oxytetracycline for control of gaffkemia in the American Lobster (Homarus-americanus). Fish Research (Amsterdam) 5 (1) . 71 - 82.

Breen, K.; Schenker S. and Himberg, M. (1975) : Fatty liver induced by tetracycline in the rat. Gastroenterology, 69 , 714 - 723.

Brown, L.L.; Albright, L.J. and Evelyn, T.P.T. (1990): Control of vertical transmission of Renibacterium salmoninarum by injection of antibiotics into maturing female coho salmon Oncorhynchus kisutch. Diseases of Aquatic Organisms, 9 (2) . 127 - 131.

Bruno, D.W. (1989) : An investigation into oxytetracycline residues in Atlantic salmon (Salmo-salar). Journal of Fish Diseases, 12, 77 - 86.

Clifton-Hadley, R.S. and Alderman, D.J. (1987): The effects of malachite green on proliferative kidney disease of salmonid fish in England and Wales. Veterinary Record, 119 , 305 - 306.

Fawcett, J. K. and Scott, J.E. (1960) : Enzymatic determination of urea nitrogen (urease modified Berthelot reaction) Jounal of Clinical Pathology, 13 , 156 - 158.

Fletcher, D.J. (1984) : Plasma glucose and plasma fatty acid levels of Limanda limanda (L.) in relation to season, stress, glucose loads and nutritional state. Journal of Fish Biology, 25 (6) , 629 - 648.

Hoffman , G. (1974) : Control and treatment of parasitic disease of freshwater fish. Fish Division of Fishery Research , Washington . D.C. pp. 7.

Huizinga, H.W.; Esch, G.W. & Hazen, T.C. (1979) : Histopathology of red-sore disease (Aeromonas

hydrophila) in naturally and experimentally infected large mouth bass (Micropterus salmonides). Journal of Fish Diseases, 2 (4) , 263 - 277.

Husdan, H. and Rapoport, A. (1968) : Estimation of creatinine by Jaffe reaction A comparison of three methods.Clinical Chemistry, 14 , 222 - 238.

Jaffe, B.M. and Behrman , N.R. (1974) : Methods of hormone radioimmunoassay. Academic Press, New York.

Kawauchi , H.; Suzuki, K.; Itoh, H.; Swanson, P.; Naito, N.; Nagohoma, Y. Nazaki , M.; Nakai , Y. and Itoh, S. (1989): The duality of teleost gonadotropin. Fish Physiology and Biochemistry, 7, 29 - 38.

Krovacek, K.; Peterz, M.; Faris , A. & Mansson, I. (1989) : Enterotoxigenicity and drug sensitivity of Aeromonas hydrophila isolated from water well in Sweden; A case study. International Journal of Food Microbiology, 18 , 144- 154.

Kudo, S. and Yazawa, S. (1997) : Binding of antibiotics to glycoproteins of the vitelline and fertilization envelopes cherry salmon eggs.

Histochemistry Journal , 29 (8) , 607 - 616.

Kusser, W.C.& Newman, S.G. (1990) : Detection of oxytetracycline residue in fish tissues using a sensitive bioassay. Journal of Fish Disease, 13 (6) , 545 - 548.

Leung, K.Y.; Lim, T.M. and Sin, Y.M. (1996) : Morphological changes in carp epithelial cells infected with Aeromonas hydrophila. Journal of Fish Diseases, 19 , 167 - 174.

Li, A.; Zhu , X. and Lu, Q. (1993) : Acute and subacute toxicity of three antimicrobial drugs on grass carp (Ctenopharyngodon idellus). Transaction Research of Fish Diseases. 12, 19 - 26.

Lorian, V. (1980) : Methods for determination of protein binding. Antibiotic in Laboratory Medicine, pp. 265 - 297 (Williams & Wilkins), Baltimore, MD.

Racicot, J.G.; Gaudet, M. and Lary, C. (1975) : Blood and liver enzymes in rainbow trout, (Salmo gairdneri Rich.) with emphasis on their diagnostic use : Study of CCl_4 toxicity and cases of Aeromonas infection. Journal of Fish Biology, 7, 825 - 835.

Reitman, S. and Frankel, S. (1957) : A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28 , 56-60.

Rintamaki-Kinnunen, K. and Valtonen, E.T. (1997) : Epizootiology of protozoans in farmed salmonids at northern latitudes. International Journal of Parasitology, 27 (1) , 89 - 99.

Sarma, D.K. (1990) : Sensitivity of Aeromonas hydrophila to antibiotics. Diseases of Aquatic Organisms, 2, 101 - 108.

Thune, R.L.; Stanley , I.A. & Cooper , R.K. (1993): Pathogenesis of Gram-negative bacterial infection in warm-water fish. Annual Review of Fish Diseases. (ed. by M.Faisal), pp. 145 - 185, Pergamon press, Oxford.

Trinder, P. (1969): Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology, 22, 158 -161.

Ueno, R. &Aoki, T. (1995) : A simplified determination of oxytetracycline in serum and muscle of culture eel by high- performance liquid

chromatography. Fish Pathology, 30 (3) , 239 - 240.

Uno, K.; Aoki, T. & Ueno, R. (1992) : Pharmacokinetic study of oxytetracycline in culture rainbow trout, amago salmon and yellowtail. Bulletin of the Japanese Society of Scientific Fisheries. 58 (6), 1151 - 1156.

Xing, S.; Lekan, S.Z. and Diczfalusy, U. (1983) : Validation of radioimmunoassay for estradiol 17-B by isotope dilution-mass spectrometry and by a test of radiochemical purity. Clinica Chimica Acta, 135 - 189.

Xu, D. and Rogers, W.A. (1994) : Oxytetracycline residues in striped bass muscle. Journal of Aquatic Animal Health, 6 (4) , 349 - 354.