EFFICACY AND RESIDUES OF OXOLINIC ACID IN
OREOCHROMIS NILOTICUS FISH INFECTED
WITH ENTERIC RED MOUTH DISEASE (ERM)

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Abstract

Efficacy and residues of oxolinic acid in tissues of experimentally inoculated Oreochromis niloticus fish with Yersinia ruckeri (Y.ruckeri) have been investigated. Results of the in-vitro study revealed that the used bacterial isolate was sensitive to oxolinic acid.

The in-vivo treatment test indicated a high effectiveness of oxolinic acid when given orally in the diet at a dose rate of 10 mg/kg body weight for ten successive days.

The haematological investigations in experimentally infected fish proved a decrease in some blood parameters namely, RBCs, WBCs count, Hb content and P.C.V. volume pre-treatment with oxolinic acid, but they returned to their normal values post-treatment. The residual analysis indicated the presence of oxolinic acid residues in the muscles, kidney and liver of treated fish up to 15, 20 and 25 days post-treatment, respectively.

INTRODUCTION

Enteric Red Mouth disease is an acute to chronic bacterial infection which affects mainly the salmonid fishes with severe economical loss (Davies 1990). Other fish species can be affected (Inglis et al., 1993). Y.ruckeri, the etiological agent of ERM was isolated from O.niloticus fish in Egypt (Mona et al., 1997). Morbidity of ERM may reach to 100% at any age of fish, while, the mortality is usually higher in younger fish (Post, 1997). Recently, the disease caused by Y.ruckeri has been referred to as yersinosis or yersinia septicemia as the classic red mouth is not necessary seen (Inglis et al., 1993). Control of clinical cases of ERM may be medicated by antimicrobial compounds and chemotherapy. Sulphamethazine and/or oxytetracycline has been advocated (Rucker, 1966) but there is evidence that certain bacteria which are pathogenic for fish can acquire
plasmide mediated resistance to the type of drug (Aoki et al., 1977). Recent work has highlighted the value of oxolinic acid for the control of yersiniosis (Rodgers and Austin, 1982). Oxolinic acid showed high efficacy against many Gram-ve bacterial fish pathogens and it is well absorbed orally (Alderman, 1988).

The present study was planned to follow up the efficacy and tissue residues of oxolinic acid in O.niloticus fish experimentally infected with Y.ruckeri, also, to measure the changes in some blood parameter of infected O.niloticus before and after treatment with oxolinic acid.

**MATERIALS AND METHODS**

1. **Fish**

A total of 120 apparently healthy alive O.niloticus fish with an average body weight of 80±10g were collected from Nawa farm at Kalubia Governorate to be used in experimental infection. Fish were kept in glass aquaria (60x80x100 cm) supplied with aerated chlorine-free tap water at 25±1°C (Inness, 1996). Fish were fed commercial ration twice a day to satiation and left 2 weeks for acclimatization. Thirty fish from each were used for determination of LD50, while, the remained 90 fish were used in experimental infection.

2. **Bacterial isolate**

   1. In this study, a complete identified locally isolated Y. ruckeri (Mona et al, 1997) was used. It was propagated on trypticase soy broth to be used in the experimental inoculation of fish and on trypticase soy agar (oxoid) to be used in the vitro sensitivity testing.

   2. *Bacillus subtilis*

      This was used as standard organism (obtained from Animal Health Research Institute) for detection of oxolinic acid residues in fish tissue.

3. **Drug**

   1. Oxolinic acid : (Urotrate), Obtained as tablets containing 750 mg oxolinic acid Park-Davis, France.

   2. Tricaine methane sulphate: (Ms-222) Sandoz, was obtained as powder and was used as fish anaesthetic at a dose of 50 mg/L (Stoskopf, 1993).
4. The in-vitro inhibitory activity of oxolinic acid

According to Austin, et al. (1981), the isolate of *Y. ruckeri* used was incubated for 7 days at 22°C on tryptone soy agar (oxoid) supplemented with 0.5, 1.0, 1.5, 10,50 and 100 mg/L of oxolinic acid for determination the in vitro inhibitory activity of oxolinic acid.

5. Preparation of medicated feed

a. Oxolinic acid was used at a rate of 10 mg/Kg body weight for treatment of *O. niloticus* experimentally infected with *Y. ruckeri* (Rodgers, et al., 1983).

b. The pellets used as fish feed were ground and thoroughly mixed with determined dose of oxolinic acid, then, wetted with water and remanufactured into suitable size pellets using pellets using pellet machine. (El-Banna, 1991).

c. Fish were fed a ration of 3% of their body weight (Meske, 1985) twice daily.

6. Detection of oxolinic acid residues

Tissue samples were assayed by the microbiological method for estimating oxolinic acid qualitatively (Levetzow, 1971.). Pieces of liver, muscle and kidney of both treated and control fish were placed over the surface of nutrient agar media inoculated with *Bacillus subtilis* spore as standard organism at antibiotic medium No. 1 (El Nasr Pharmaceutical Chemical Co.). The inhibition zone around the tissue samples was measured; 2mm or more was considered positive result indicating the presence of antibiotic residue, 1mm or less was considered negative result indicating the absence of antibiotic residue (Arret et al. 1971).

7. Determination of LD<sub>50</sub> of *Y. ruckeri* isolate

This was applied according to Reed and Menuch (1938). Thirty *O. niloticus* fish, were divided into 6 groups (5 fish/group). Fish in each group were injected i/M by 0.1 ml of bacterial dilution, 10<sup>1</sup>, 10<sup>2</sup>, ..., 10<sup>6</sup>, of which the bacterial cell count of original sample was 2.5x10<sup>8</sup> cell/ml.

8. Experimental design

In this study, a total of 90 apparently healthy normal *O. niloticus* with an average body weight (80±10 g) were divided into 3 groups of 30 fish each. All fish were anaesthetized and fish in group 1 and 2 were injected sub/cut with 0.5 ml of 24 h old broth culture containing 2x10<sup>2</sup> cell/ml of *Y. ruckeri*, while, each fish in
group 3 was injected sub-cut with 0.5 ml/fish of sterile saline and kept as control. Fish were observed for the development of clinical signs indicating *Y. ruckeri* infection. After the appearance of clinical signs in experimentally infected group, (72 h post-infection), ten blood samples were randomly collected from each group for haematological examination according to Lucky (1977). After that, the fish in the fist group were fed on medicated diet (10 mg of oxolinic acid/Kg body weight) for 10 days according to Collins *et al.* (1996), while, fish in the other groups were fed on medicated free diet, 72 h after the end of treatment. Other 10 blood samples were randomly collected from the first group. Blood samples were used for estimating total erythrocytic counts, packed cell volume (Haematocrite) haemoglobin percentage and total leucoytic counts according to the method described by Lucky (1977).

For studying the residues of oxolinic acid, 3 samples were taken from muscles, kidney and liver from fish in group 1 after killing at the rate of 5 days intervals through 30 successive days. A positive result was indicated by the complete-inhibition of growth of the tested organism in anular zone not less than 2 mm width (Levetzew, 1971 and Anon, 1980).

**RESULTS AND DISCUSSION**

The results of LD$_{50}$ of *Y. ruckeri* isolate carried out according to Reed and Menuch (1938), indicated that LD$_{50}$ value for *Y. ruckeri* isolate was 2.5x10$^6$ cell/ml. Studying the in-vitro inhibitory activity of oxolinic acid revealed that the *Yersinia ruckeri* isolate was sensitive to 5 mg/l. These findings concide with Austin, *et al.* (1981) and Collins, *et al.* (1996).

**Efficacy of oxolinic acid**

The most pronounced clinical signs of the experimental *Y. ruckeri* infection on *O. niloticus* fish started through the first 48 h post-infection. Infected fish showed sluggishness and darkness in colour (Fig. 1), petechial haemorrhage or haemorrhagic body surface, tail and fins be eroded and erythema in the mouth region. The moribund fish percentage was 60% in the first two groups (infected groups), while, mortality rate was low (10%) (Table 1). In case of group 1 (treated group), clinical signs indicating *Y. ruckeri* affection started to relieve within 96 h post-fed on medicated diet with oxolinic acid. Fish returned to their normal activity, signs of septicemia declined, the mortality rate was stopped. During the reisolation, *Y. ruckeri* disappeared from internal organs of treated fish, while, it was isolated from internal organs from non-treated fish (group 2), in which the lesions progressed to haemorrhagic patches all over the body, particularly around the base
of the fins and in the head regions (Fig. 2). In some fish, the anus was protruded and oedematous with redness in the perianal tissue. At the end of experiment, the morbidity rate reached to 90%, while, mortality reached 40%. These findings coincide with those of Wobsere (1973). Results of in-vitro study confirmed the previously mentioned results of the effect of oxolinic acid on infected fish with *Y. ruckeri*.

**Haematological findings**

It appears from Table 2 that, the experimental infection of *O. niloticus* with *Y. ruckeri* resulted in significant decrease in red blood cell counts, haematocrit values, haemoglobin content and total leucocytic count. These findings were also in agreement with those reported by Quentel and Aldrin (1986) and Lehmann et al. (1987). Miller (1983) suggested that the endotoxin of *Y. ruckeri* could affect blood coagulation, ultimately producing thrombosis in the capillaries and haemorrhages. However, all examined parameters approached to their normal limits after the course of medicated feed, but they still showed non-significant decrease than their normal limits.

**Oxolinic acid residues**

The residues of oxolinic acid in experimentally infected fish group fed on the medicated ration were determined qualitatively as shown in Table 3. It is clear that, oxolinic acid residues were detected in muscles up to 15 days, in kidney up to 20 days and finally up to 25 days in liver post-treatment. The presence of drug residues in kidney and liver more than muscles may be attributed to the fact that, kidney is the last gate allowing passage of drug before excretion, and liver plays the major role for detoxication and storage of the drug.

It could be concluded that, the use of oxolinic acid in ration can overcome the carrier state of *Y. ruckeri* which serves as an important source of infection under stress condition. We can also conclude that, fish treated with the drug should not be used by the consumer before 25 days following treatment to avoid resistance.
Fig. 1. *Oreochromis niloticus* experimentally infected with *Y. ruckeri* 96 h. post-infection showing darkening in colour.

Fig. 2. *Oreochromis niloticus* experimentally infected with *Y. ruckeri* 5 days post-infection showing redness around mouth, eyes and perianal tissue.
Table 1. Effect of oxolinic acid on the morbidity and the mortality rate of *O. niloticus* 
sub/cut injected with 0.5 mmol of 2x10^6 cell/ml *Y. ruckeri*

<table>
<thead>
<tr>
<th>Fish groups</th>
<th>Type and duration of treatment</th>
<th>Morbidity percentage</th>
<th>Mortality percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
</tr>
<tr>
<td>1 (treated)</td>
<td>fed on diet contain 10 mg oxolinic acid / body weight for 10 days</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>2 (non treated)</td>
<td>fed on diet free from oxolinic acid</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>3 (control)</td>
<td>fed on diet free from oxolinic acid</td>
<td>-</td>
<td>-</td>
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</tbody>
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Table 2. Haematological picture of *O. niloticus* fish infected with *Y. ruckeri*, before and after medication with oxolinic acid.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Haematological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBCs 10^6/mm³</td>
</tr>
<tr>
<td><em>infected fish before treatment</em></td>
<td>1.1 ± 0.20*</td>
</tr>
<tr>
<td><em>infected fish 10 day after medicated ration</em></td>
<td>1.84 ± 0.62</td>
</tr>
<tr>
<td><em>Control</em></td>
<td>1.9 ± 0.22</td>
</tr>
</tbody>
</table>

Significant at P<0.05.
Table 3. Oxolinic acid residues in *O. niloticus* fish.

<table>
<thead>
<tr>
<th>Groups</th>
<th>treated sample</th>
<th>Days post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>Muscle</td>
<td>3/3</td>
</tr>
<tr>
<td>treated group</td>
<td>Kidney</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3/3</td>
</tr>
<tr>
<td>Control</td>
<td>Muscle</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0/3</td>
</tr>
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</table>

The results are expressed as the number of samples with positive inhibition in relation to the number examined.
REFERENCES


كفاءة ومتتابقات حامض الأوكسولينيج في علاج أسماك البلطي المصابة بجريبيا برميروكوب البروسينيا روكرى
منى حصين و عباس يونس

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تعد دراسة تأثير وبقايا حامض الأوكسولينيج في سمك البلطي المصاب برميروكوب البروسينيا روكرى محروماً.
وقد أظهرت الدراسة أن مستويات الدراسية كانت عند السمك في معظم الظروف. استغرقت الأمراض المرتبطة في الأسماك المصابة بجربيا برميروكوب البروسينيا روكرى، ويقياس عند كرات الدم الحمراء، وبياضة، ونسبة الهيموجلوبين، ونسبة الترسيب الدموي في الأسماك المصابة، مما يعكس عن معدلات الطبيعية أثناء الاشبة بالحروق التجريبية وعادت إلى طبيعتها بعد استخدام العلاج. وأيضاً أثبتت الدراسة أن مستويات السمك المقرر أن من اجهاض الأسماك المطبوخ بعد 15 يوماً من العلاجات الكافية والكبد على التركيب.