Estimation of skin and gill monogenea and musculature in *O. niloticus* treated by praziquantel using HPLC

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Abstract

Treatment of skin and gill monogenea in *O. niloticus* with the effective drug, praziquantel which used in eradication of most helminthes in human, animals and fish. It used also in treatment of schistsomes in human being. The levels of praziquantel were determind in skin and gills (for treatment skin and gill monogenea) and musculature of *O. niloticus* (for determination of withholding time) with a bath treatment at 100 ppm of praziquantel for 15 minutes after 5, 10, 24, 48, 72, 96, 120 and 144 hours sampling time using HPLC. Praziquantel was highest at the 48 hours in skin and at 24 hours in gills and 10 hours in musculature (after sampling time), and it was depleted after 120 hours, 72 hours and 72 hours in skin, gills and musculature respectively in *O. niloticus*. [Life Science Journal. 2008; 5(3): 77 – 82] (ISSN: 1097 – 8135).

Keywords: skin and gill monogenea; *O. niloticus*; praziquantel; musculature

1 Introduction

Monogenean parasites have been recognized as a serious pathogen of fish in aquaculture (Ogawa, 2002; Whittington *et al*, 2001; Ernst *et al*, 2002; Grau *et al*, 2003; Montero *et al*, 2003; 2004). Monogeneans are able to multiply rapidly in high density aquaculture environments because they have a direct single host life cycle as they require no intermediate host (Rohde, 1993) and they produce freely deposited eggs that often become entangled to high re-infection rates amongst fish (Ernst *et al*, 2002; Ogawa, 2002).

Farmed fish frequently develop heavy infestation with monogenean parasites, resulting in epithelial hyperplasia of gill lamellae. Severely affected fish may die, as a result of gill pathology and interference with the exchange of respiratory gasses and ions (Whittington *et al*, 2002; Stephens *et al*, 2003).

There are two common of freshwater flukes, gyrodactylus and dactylogyrus which differ markedly in their reproductive methods as well as their preferred attachment sites on host fish.

Gyrodactylus are generally found on the body and fins of fish. Adult parasites carry a fully developed embryo identical to the adult, which in turn carry young of next generation. Therefore, each individual parasite may represent several generations (Woo, 2002; Eissa, 2002).

One chemical treatment may be adequate to control an infestation while members of genus dactylogyrus prefer to attach to gills of host fish and unlike gyrodactylus are egg layers. Eggs produced by the adults fall to the bottom of aquarium where they accumulate in organic debris in the gravel (Umeda *et al*, 2006). The eggs can be resilient to chemical treatment, which make the use of multiple chemical treatments appropriate to control this group of organisms.

There are few studies on the control of fish parasites and even fewer effective or accepted methods for the direct or indirect control of these parasites .

Several treatment regimes have been used however, control of the parasite has been difficult because many treatments are either of high risk to fish or not effective in controlling the parasite (Szekely and Molnar, 1990; Toney, 1990).

Thus praziquentel has been the treatment of choice to

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control various external and internal helminthes infections in fish and human being. It is an anthelminic used in most schistosome treatment for human being in Egypt under commercial name (Biltricid), many cestodes and trematodes infestations (Schmal and Melhorn, 1985; Buchmann *et al*, 1990; Buchmann, 1999). Praziquantel affects the permeability of the cell membrane of the prasites resulting in increased contraction of prasites, the drug further causes vacuolization and disinteration of monogenea teguments (Schmahl and Taraschwski, 1987). It was used at 100 mg/L as a bath for 3 – 15 minutes to control both gills and body flukes and has a wide margin of safety for fish. Prazequentel is toxic to flukes on contact, paralyzing the parasites within 15 minutes. It must be dosed high and long enough for effective treatment. So, it is important to medicate long enough to intercept the emerging larvae (Schmal and Melhorn, 1985).

Monitoring tissue gills and skin drug concentration at the site of parasite activity (predilection site) post treatment can provide a useful mechanism for determining how and when treatment efficiency may be optimized. Furthermore it may be possible to adjust the dose in an effort to improve the efficacy of treatment.also determination the withholding period of praziquantel in *Oreochromis niloticus* (*O. niloticus*) musculature for safely consume the fish without prziquantel muscle residues (Kim *et al*, 2003; Tubbs and Tingle, 2006).

Therefore the present study aimed to investigate the effect of bath treatment for controlling gills and skin monogenea in *O. niloticus* and determine the highest level of praziquentel in gills and skin and determination of depletion period of praziquantel in musculture of *O. niloticus* to avoid the drug resistance, using reversed phase of high performance liquid chromatography (HPLC) and diazepam was used as internal standard.

2 Materials and Methods

2.1 Fish

Forty, clinically healthy *O. niloticus* weighing 80 – 100 g were obtained from private fish farm. The fish were acclimatized for 14 days before experiment. The fish were starved during both the acclimatization and the experimental period to avoid differences in drug kinetics owing to differences in nutritional status.

Bath treatment for fish at acocentration 100 ppm for 15 minutes.

2.2 Chemical reagents

Praziquentel that is 2-cyclohexylcarbonyl-4-oxo-1,2,3,6,7,11 b-hexahydro-4H-pyrazino [2,1-a] isoquinline and the internal standard, diazepam that is 7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepine-2[1H]-one were donated by Alexandria company and Nile company for pharmaceuticals and chemical industries, Cairo, Egypt.

Acetonitrile for mobile phase and distilled water were of chromatographic grade.

Standard solutions of praziquentel were made by dilution of stock solution (10 μg of praziquentel/ml of mobile phase) the internal standard solution was prepared by dissolving 10 μg of diazepam into 1 ml of mobile phase.

2.3 Chromatographic conditions

The instruments used were a Hewlett-packed (HP 1100 Series, USA) HPLC equipped with QUAT pump (HP 1100 Series G132A), an injection valve fitted with 5 ml sampling loop, a variable wavelength UV detector and a data module. Analysis was performed on an ODS2, C18 column (125 mm × 4 mm, Hewlett-Packard) with acetonitrile-water (45 : 55, V/V) as the mobile phase. The column was kept at room temperature (20 – 24 ºC) at the flow rate was kept constant at 1.0 ml/minutes. The detector wavelength was set 217 nm. Between each 200 μl injection, the column was washed for 30 minutes with 100% acetonitrile. The detection limit of the assay was 0.05 μg/g in both skin and gills.

2.4 Samples preparation

Two grams portion of gills, skin or muscle were weighed into a 15 ml corning tube, and 8.6 ml of 100% acetonitrile was added.

The sample was ground using a homogenizer (ART-Moderne Labortechnik, Miilleim, Germany), and then 0.4 ml of internal standard solution was added.

After allowing standing for 10 minutes at 4 ºC, the sample was centrifuged at 10000 × g for 10 minutes and the supernatant was collected. The collected supernatant was evaporated to dryness with a speed vacuum (Heto-Holten, Copenhagen, Denmark). The dry residue was dissolved in 1 ml of mobile phase and a portion of 175 μl was injected into the HPLC.

2.5 Calibration curve

Skin and gills homogenate were spiked with standard solution of praziquentel and internal standard to yield concentrations of 0.5, 1.0, 2.5, 5.0 and 10.0 μg/ml of praziquentel and 4.0 μg/ml of diazepam. The sample was conducted as the above procedure, and each level was assayed in triplicate.

2.6 Bath treatment

Aquarium fish were bathed in an contaning aconcentration of 100 ppm/15 minutes praziquantel during the treatment the flow through system was stopped and water aerated vigrously to maintain a high oxygen concentration and to prevent the drug to from a sediment, after 15 minutes. Fish was transfered to free chlorine freshwater with contineous aeration at 5, 10, 24, 48, 72, 96, 120 and 144 hours post treatment fish were taken randomly from the aquarium gills, skin and muscle. Samples from each fish were collected as described before.

3 Results and Discussion

Chromatograms of gills homogenate for calibration were shown in Figure 1. Linear relationship was found when the ratio of the peak hight of prazquiantel in the gills to that of the internal standard was plotted against the cocentration of praziquantel in the range of 0.5 – 4.0 µg/ml. The retention time for praziquantel and internal standard (diazepam) were 3.1 minutes and 4 minutes for skin and 2.6 and 4 minutes for gills and 3.4 and 4 minutes for musculature homogenate, respectively (Figures 2, 3 and 4).

The concentration of praziquantel found in skin, gills and musculature of *O. niloticus* after praziquantel bath treatment (100 ppm/15 minutes) are shown in Table 1. Praziquantel concentration in the skin was significantly higher than gills. Musculature praziquantel was found in skin, gill and musculature until 120 hours, 72 hours and 72 hours respectively. In skin praziquantel was highest at the 48 hours sampling time and declined gradually thereafter. The concentration of praziquantel in gills was highest at 24 hours samplimg time and declined sharply. Praziquantel concentration was highest at 10 hours in musclature of *O. niloticus* and declined with time. The praziquantel in musculature was significantly lower than those of gills and skin. The absorption of praziquantel through bath treatment was determind in *O. niloticus* by HPLC for the first time in the species of fish for determination the withdrawal time in muscles also for treatment of Gyrodactylus (skin monogenea) and Cichylidogyrus (gill monogenea) by the relatively long time taken bath

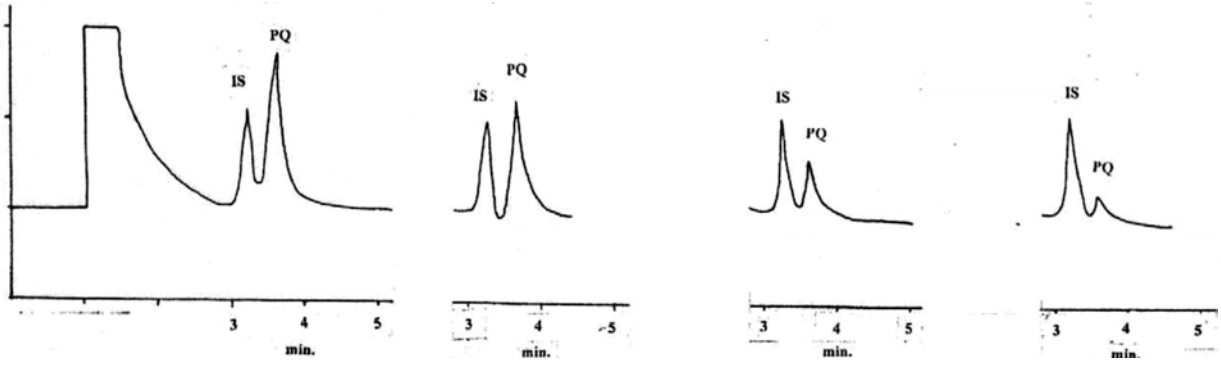

Figure 1. Chromatograms of praziquentel (PQ) determination in *O. niloticus* gills for standardization.

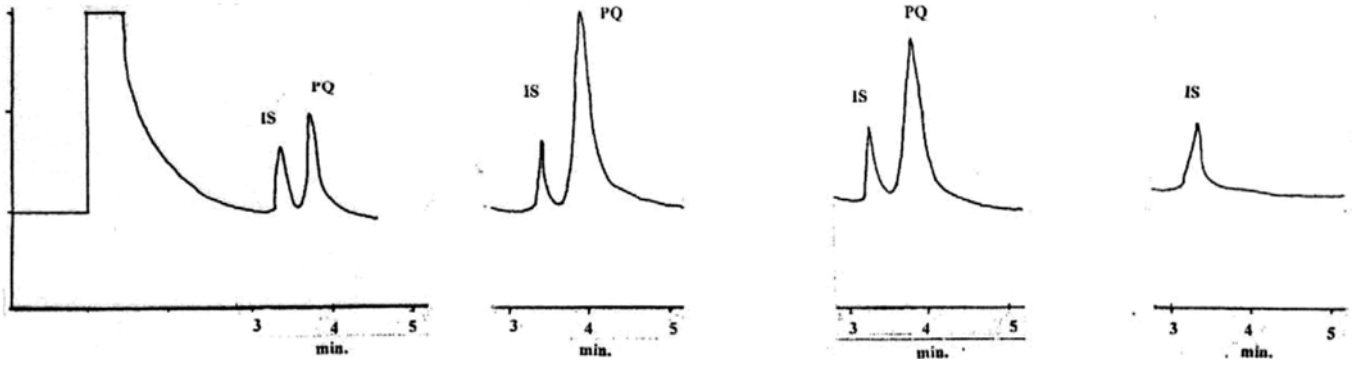

Figure 2. Chromatograms of PQ determination in skin of *O. niloticus* compared with diazepam as an internal standard (IS).

to achieve maximum tissue concentration in skin and gills. For treatment of monogenea in *O. niloticus* using bath 100 mg/L for 15 minutes of praziquantel as bath treatment, the study appears that the accumulation of the drug in a very limited mannar and sharply declined in skin, gill and muscle of *O. niloticus*. This is probably due to rapid clearance by either hepatic metabolism and renal excretion (Kim *et al*, 2001; 2003) (Table 1).

This study demonstrates the advantges of pharmacokinetic approach, which can with it adjust the dose regimens in treatment monogeniasis in *O. niloticus*. Important imformations were revealed about drug accumulation and question then be asked whether the accumulation may in fact become of a negative or a postive effect

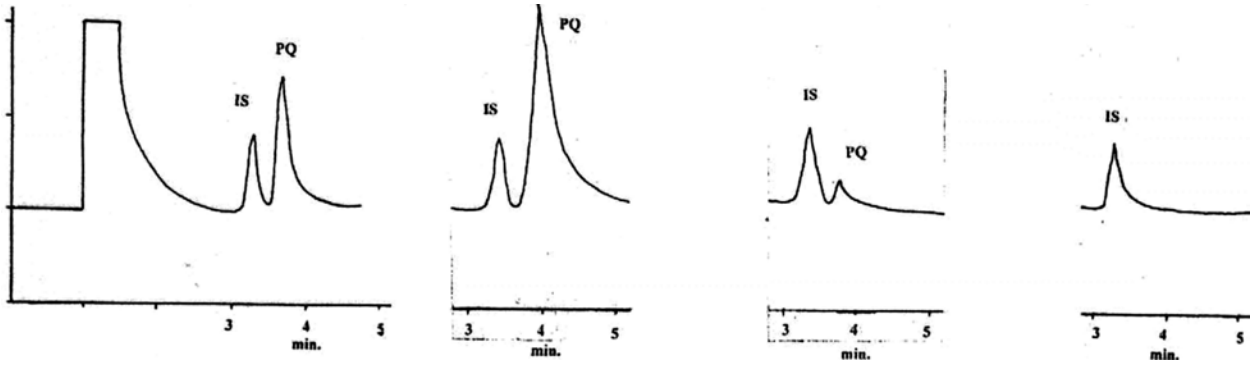

Figure 3. Chromatograms of PQ determination in gills of *O. niloticus* compared with diazepam as an IS.

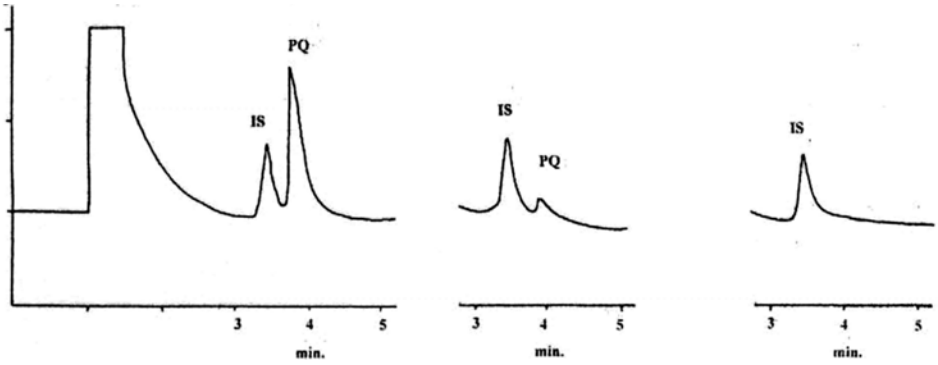

Figure 4. Chromatograms of PQ determination in musculature of *O. niloticus* compared with diazepam as an IS.

Table 1. *O. niloticus* skin, gills and musculature after bath treatment with praziquantel (100 ppm/15 minutes)

Sampling time after treatment (hours)	Skin (mg/g)	Gills (mg/g)	Musculature (mg/g)
5	1.75 ± 0.15	3.38 ±1.50	0.20 ± 0.04
10	2.25 ± 0.92	3.93 ± 1.23	1.10 ± 0.50
24	4.03 ± 0.45	4.56 ± 0.78	0.92 ± 0.22
48	5.27 ± 3.22	2.00 ± 0.92	0.21 ±0.06
72	3.33 ± 1.12	0.94 ± 0.24	0.04 ± 0.07
96	1.90 ± 0.42	Nd	Nd
120	0.87 ± 0.28	Nd	Nd
144	Nd	Nd	Nd

Nd: not detected.

under existing bath treatment of *O. niloticus* monogeniasis which will expose the fish to high concentration of praziquantel through treatment, also bathing interval is benefit by forming restricted manner for treatment the delivered larvae of Gyrodactylus and the hatched of Cichildogyrus. Potential benefits of this approach include minimizing wast time and cost associated with the drug adminstration as well as minimizing the potential development of antihelmintic resistance which caused by extended exposure of parasites to subcurative doses (Kim *et al*, 1998; Kim and Cho, 2000; Tubbs and Tingle, 2006) similar studies were made. For other species of fish represented as rockfish, kingfish, salmon and rainbow trout but the present study carried out on the Egyption cultured *O. niloticus*, they were studied the effect of praziquatel accumulation in plasma, skin musculature and other tissues (Rogstad *et al*, 1987; Hormazabal and Yndestad, 1995) and demonstrated the minimal accumulation of adminatrated (oral and bath) praziquantel in their fish species (Kim *et al*, 2001; 2003; Tubbs and Tingle, 2006).

The lack of accumulation may be beneficial by reducing the with holding periods of treated fish by praziquantel because the use of praziquantal in food fish may lead to residues in fish tissues, and public health authorities require safe drug withdrawal periods (Kim *et al*, 2001) .

The present study determine the withdrawal period of praziquantel in musculature of *O. niloticus* and indicated that the maximum concentration of praziquantal in musculature recorded after 10 hours post treatment then sharply decreased and eliminated after 96 hours, thus *O. niloticus* can safely used for human being after 96 hours of bath with praziquantal in concentration 100 ppm for 15 minutes, while the maximum level of praziquantal in musculature of rockfish was after 3 hours then decreased sharply and eliminated from musculature after 48 hours because the bath in the present study was 100 ppm/15 minutes while the bath of the rockfish was 100 ppm/4 minutes. Thus the difference of elimination and highest concentration time may be due to the difference of bath time and species difference. Considering the sharp decrease of praziquantal concentration in skin, gills and muscle after 120 hours, 72 hours and 72 hours respectively after bath treatment (100 ppm/15 minutes) the benefits in retreatment at an interval of 120 hours to be more effective for eradication of delivered larvae of gyrodactylus and hatched larvae of cichylidogyrus which inhabit skin and gills. The appearance of higher praziquantel skin concentration may be due to the extremely lipophilic nature of praziquantel which may cause the drug to partition more into the fatty tissues underlying the hypodermis (Tubbs and Tingle, 2006). It is known that the gills is the main route of absorption for adminsration in water (Treves-Brown, 2000) however direct absorption through the skin cannot be excluded. Bathing *O. niloticus* with 100 ppm/15 minutes in the present study resemble that of the scheme of treatment of gyrodactylus and cichylidogyrus in the field condition (Schmahl and Taraschwski, 1987; Ngim Eldin and Saleh, 1995; Stephens *et al*, 2003; Osman, 2005; Bobadilla *et al*, 2006) regarding the praziquantel residue concentration in musculature of *O. niloticus* was about 8 times lower than that in the skin and 16 times than those of the gills. These results suggests that absorption of praziquantel in *O. niloticus* following bath treatment is largely throguth gills, but small amount of the drug can be absorped througth scaly skin of *O. niloticus*. Although the cocentration of praziquantel in gills was lower than that of the skin it affect not only with direct contact with monogenea in skin and gills but also by feeding the parasites on blood tissues of gills which contian the drug, resulted in high treatment efficacy.

From the pervious results we can conclude that using of information about the accumulation and witholding period of praziquantel in *O. niloticus* for treament skin and gill fluks and rebathing after 120 hours for complete eradication of deliverd and hatched larvae also the study highlights on the depletion period of praziquantel in *O. niloticus* musculature guiding for safety using after 96 hours, advising for making simillar studies on other Egyptian fish species.

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