

The Effect of Praziquantel on Selected Haematological and Biochemical Indices in Nile Tilapia (*Oreochromis niloticus*) with Tapeworm Infestation

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ABSTRACT

The study was planned to assess the effects of the antihelmintic, Praziquantel, on haematological and biochemical indices in blood of Nile tilapia (*Oreochromis niloticus*). Fish were divided into 3 groups: first group received 40 mg Kg⁻¹ body weight (bw) Praziquantel, and second group were given 60mg Kg⁻¹ body weight (bw) praziquantel mixed into special prepared fish ration (private factory). Fish were examined every 48 hours after administration. Praziquantel treatment was characterized by scientifically lower erythrocyte count, haemoglobin level, packed cell volume and total protein both dose levels after 48 and 96 hours compared to the third control group. The activity of alanine aminotransferase (ALT) was scientifically higher after 48 and 96 hours in treated groups, which could be attributed to slight hepatocyte damage caused by praziquantel. On the same manner the lysozomal activity and phagocytic activity and antibody titer against *Aeromonas hydrophila* were lower than control values after 48 and 96 hours.

Keywords: Praziquantel, Haematological and Biochemical Indices, Nile Tilapia.

INTRODUCTION

Chemicals used in aquaculture are subjected to strict control, particularly with regard to

their safety and efficacy (Taylor and Roberts, 1999). One of the important categories of widely used veterinary therapeutics is antihelmintics. An effective fish

anthelmintic (useable in food fish) must meet a number of general requirements, e.g. it must be non-toxic to both fish and humans, have a large safety factor without side effects, and leave no residues in fish (Roos et al. 2001).

Praziquantel is a parasiticide which is effective against tapeworms, monogeneans, and trematodes (Kim et al. 2001 and Sharp et al. 2004). It was the first anthelmintic to fulfil the WHO requirements for population-based chemotherapy for a broad range of parasitic infections (Wegner, 1981). The chemical entity was discovered in 1972. It was developed first for the veterinary market and then for treatment of schistosomiasis in humans (Reich and Govindaraj, 1998). Optimum dose regimens and use conditions have not been determined in most cases. The effective doses cover a wide range: 5-100 mg kg⁻¹day⁻¹ depending on species of tapeworm (Treves-Brown, 2000).

Veterinarians have attempted to use medication for the treatment of fish tapeworm, but little information on their effects has been reported. Various

chemicals, including praziquantel, benzimidazoles, and niclosamides, have been recommended for oral treatment of fish tapeworm (Treves-Brown, 2000), but there are no determined maximum residue limits (MRL) of these substances in fish to be used for human consumption. At present, praziquantel is licenced for use as a veterinary prescription drug only in ornamental and non-food fish (Kolarova and Nepejchalova, 2006).

Blood parameters are considered as pathophysiological indicators of the whole body and therefore are important in diagnostic the structural and functional status of fish exposed to toxicants and medicaments (Adhikari et al. 2004). Therefore, the aim of the present study was to assess the effects of Praziquantel on the haematological indices and biochemical blood profile of *Oreochromis niloticus* with parasitic infestation.

MATERIALS AND METHODS

Fish husbandry

110 Nile tilapia, mean body weight 80 ±5 gm, some of which

were naturally infected with tapeworms, were collected from a fish farm in Behera provinince. The fish were acclimated for one week and were fed twice daily with dry pelleted food. Feeding was discontinued 24 h prior to beginning the experiment. The health of fish, prevalence and intensity of infection were established through examination of 10 fish prior the trial.

90 Nile tilapia were divided into 3 groups of 3 replicates of each group, each replicate contained 10 fish, 30 fish were used as a control. The volume of each experimental aquarium was 50 L and water was changed daily. Water was aerated and oxygen saturation and pH were monitored daily. Oxygen saturation of water was above 85% (ranging from 80 to 90%) and pH ranged from 6.3 to 7.3. Water temperature was checked 6 times daily, from 29 May to 12 June 2009, the mean daily water temperature was 25°C ($\pm 2^\circ\text{C}$) in all aquaria.

Praziquantel® (El-Nasr Co.) trial

Fish were fed on special fish ration with varying concentrations of drug mixed into full prepared

ration. The experimental group A were given 40 mg Praziquantel (2 - cyclohexylcarbonyl - 1 - 4 - oxo - 1,2,3,6,7, 1 Ib - hexahydro - 4H - pyrazino [2,1 - a] isoquinoline) kg^{-1} body weight (bw). Experimental group B were treated with 60 mg praziquantel kg^{-1} bw. The fish in the control group C received only ration without the drug. The fish were subsequently observed in the recovery aquaria for detecting any signs of regurgitation of anthelminthic.

Parasitological examination

Parasitological examination was carried out according to Paperna (1986). The internal wet smear made by small scissor at the injection between (stomach and intestine). Then by cover slide make scraping the internal edge of the opened part.

Blood sample processing

Blood was taken 48 and 96 h after drug administration in groups A, B and C. Blood was sampled from the caudal vessels and stabilized with 50 IU sodium heparin per 1ml. Immediately after sampling, erythrocyte count (RBC), haematocrit value (PCV),

haemoglobin concentration (Hb), leukocyte count (WBC), mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined according to Svobodova et al. (1991). Plasma was separated from cells by centrifugation (10 min at 12 000 x g) at 4 °C. The biochemical indices determined in plasma included glucose (Glu), total plasma protein concentration (TP), total globulins (Glob), and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). For the biochemical analysis of plasma, a VETTES 8008 Analyser (IDEXX Laboratories, Westbrook, ME, USA) was used. After blood sampling, the fish were quickly stunned with a blow to the head and examined for presence of tapeworms in the intestine. Evaluation of potency of prepared vaccine against *Aeromonas hydrophila* according to the method described by Newman and Majnarich (1982) and Badran (1990).

Statistical analysis

The statistical software program STATISTICA (version 8.0

for Windows, StatSoft) was used to compare differences among the test groups. Prior to analysis, all measured variables were checked for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance (Bartlett's test). If those conditions were satisfied, a two-way ANOVA was employed to determine whether there were significant differences in measured variables among experimental groups. When a difference was detected ($P < 0.05$), Dunnett's multiple range test was applied (Zar, 1996).

RESULTS

Clinical picture of treated fish

No clinical abnormalities were observed in the fish treated with praziquantel at either dose level. Praziquantel and non-treated groups were well tolerated by the fish, and no fish were lost during the experiment. The signs appeared. (Fig. 1)

Haematological profile

The most significant differences from controls were



Fig. (1): Fish (*O.niloticus*) with parasitic infestation.

observed in haematological indices 48 and 96 h after administration. In the A and B groups, significantly lower values ($P < 0.01$) of RBC and Hb compared to group C were observed.

The value of PCV was significantly lower in the groups A and B ($P < 0.01$, $P < 0.05$, respectively) compared to group C. The response of WBC to praziquantel was less dramatic. The WBC counts were similar for all groups at both sampling times. The basic values of RBC -MCV, MCH and MCHC also showed no significant differences among groups throughout the experiment (Table 1 and 2).

Biochemical profile of blood plasma

There were no observed differences from controls in ALT

activity after 48 and 96 h. While AST did not differ from control at either sampling times. In Glu values, there was a significant reduction ($P < 0.05$) in groups A and B when compared with C (after 48 and 96 h). The values of TP and Glob were significantly lower ($P < 0.05$) in group A after 48 h compared with C. In the group B, we found also lower values of TP and Glob, however, they were significant ($P < 0.05$) compared with control only in Glob. The values of biochemical indices for all groups are given in (Tables 3 and 4).

Effect of praziquantel on antibody titration and relative level of protection

The antibody titration differed significantly among different groups ($P < 0.01$) according to the

PRAZIQUANTEL HAEMATOLOGICAL AND BIOCHEMICAL INDICES IN NILE TILAPIA

Table (1): Effects of praziquantel 48 h post administration, on haematological indices in *O.niloticus*. Groups with different alphabetic superscripts differ significant!) at $p < 0.05$ or $p < 0.01$.

Indices	C	A	B
	X± SD (n=10)	(40 mg kg ⁻¹) X± SD (n=10)	(60 mg kg ⁻¹) X± SD (n=10)
RBC (T.1 ⁻¹)	1.87 ±0.27 ^a	1.46 ±0.34 ^b	1.45 ±0.20 ^b
Hb (g.1 ⁻¹)	107.50 ± 12.62 ^a	81.80 ± 20.36 ^b	81.70 ± 10.84 ^b
PCV(1.1 ⁻¹)	0.35 ± 0.04 ^a	0.23 ± 0.04 ^c	0.20 ± 0.04 ^c
WBC (G.1 ⁻¹)	58.2±19.92 ^a	57.65±20.43 ^a	49.50±15.20 ^a
MCV (fl)	1 90.04 ± 27.63 ^a	166.42 ± 24.83 ^a	216.61 ± 53.97 ^a
MCH(pg)	57. 93± 3.92 ^a	57.03± 4.11 ^a	56.34± 3.32 ^a
MCHC (11 ⁻¹)	0.31 ± 0.04 ^a	0.30 ± 0.05 ^a	0.27 ± 0.05 ^a

Table (2): Effects of praziquantel 96 h post administration, on haematological indices in *O.niloticus*. Groups with different alphabetic superscripts differ significantly at $p < 0.05$ or $p < 0.01$.

Indices	C	A	B
	X± SD (n=10)	(40 mg kg ⁻¹) X± SD (n=10)	(60 mg kg ⁻¹) X± SD (n=10)
RBC (T.1 ⁻¹)	1.64 ±0.14 ^a	1.63 ±0.13 ^a	1.62 ±0.20 ^a
Hb (g.1 ⁻¹)	86.30 ± 7.60 ^a	85.40 ± 7.99 ^a	84.70 ± 11.28 ^a
PCV(1.1 ⁻¹)	0.29 ± 0.03 ^a	0.28 ± 0.03 ^c	0.26 ± 0.04 ^a
WBC (G.1 ⁻¹)	73.95±28.32 ^a	67.45±11.72 ^a	60.20 ±20.97 ^a
MCV (fl)	180.29± 15.30 ^a	183.87 ± 11.51 ^a	177.57 ± 12.91 ^a
MCH(pg)	52.94 ± 4.66 ^a	51.57± 2.55 ^a	50.83± 4.13 ^a
MCHC (11 ⁻¹)	0.29 ± 0.02 ^a	0.27 ± 0.02 ^a	0.26 ± 0.30 ^a

Table (3): Effects of praziquantel 48 h post administration, on biochemical indices in *O.niloticus* plasma. Groups with different alphabetic superscripts differ significantly at < 0.05 or $p < 0.01$).

Indices	C	A	B
	X±SD (n=10)	(40 mg kg ⁻¹) X±SD (n=10)	(60 mg kg ⁻¹) X±SD (n=10)
AST (U.1 ⁻¹)	64.2 ±48.80 ^a	65.2 ±24.81 ^a	67.9 ±16.06 ^a
ALT (U.1 ⁻¹)	6.2 ± 6.03 ^a	13.4 ± 8.99 ^a	13.7 ± 9.48 ^a
GLu (mmol.1 ⁻¹)	7.85 ± 1.698 ^a	8.75 ± 1.41 ^b	8.96 ± 1.547 ^b
TP (g.1 ⁻¹)	37.3±5.86 ^a	27.9±5.25 ^b	27.4 ±3.67 ^a
Glob (g.1 ⁻¹)	32.5± 5.27 ^a	25.8 ± 3.12 ^b	24.6 ± 2.62 ^b
Antibody titer	Ab 6.12±0.33	abc 5.35±0.58	bc 4.98±0.58

Table (4): Effects of praziquantel, 96 h post administration, on biochemical indices in *O.niloticus* plasma. Groups with different alphabetic superscripts differ significantly at $P < 0.05$ or $P < 0.01$).

Indices	C	A	B
	X±SD (n=10)	(40 mg kg ⁻¹) X±SD (n=10)	(60 mg kg ⁻¹) X±SD (n=10)
AST (U.1 ⁻¹)	51.7 ±17.02 ^a	55.8 ±10.44 ^a	56.2 ±7.01 ^a
ALT (U.1 ⁻¹)	13.6± 5.23 ^a	21.4 ± 6.99 ^a	22.5 ± 5.48 ^a
GLu (mmol.1 ⁻¹)	4.14 ± 0.67 ^a	4.47 ± 0.931 ^a	4.53 ± 0.58 ^a
TP (g.1 ⁻¹)	34.1±1.51 ^a	34.0±1.95 ^a	32.3 ±3.00 ^a
Glob (g.1 ⁻¹)	29.6± 0.66 ^a	28.9 ± 1.22 ^a	28.5 ± 1.86 ^a
PA (%)	23± 2.0 ^a	22.1± 2.0 ^a	20.4± 2.0 ^a
Antibody titer	Ab 7.33±0.33	abc 6.15±0.58	bc 6.00±0.58

effect of praziquantel used and period of treatment. The higher antibody titers observed in the control non-treated group, then group A and group B. At the same level RLP was higher in control +ve than group A and group B. (Tables 4 and 5).

Parasite infection

The presence of tapeworms in the intestines of both non-medicated and experimental fish was stated. (Fig. 2). Worms were identified as *Atractolytocestus huronensis*. We evaluated only the prevalence of tapeworms because of low intensity of infection. The prevalence of tapeworms was 30% in fish checked before the experiment began, and no tapeworms were found in group A after treatment (prevalence 0%).

The prevalence of tapeworms in group B was 10% after treatment. The control group C had the same prevalence (30%) as the fish checked before the trial. Yellow coloured remains of tapeworms were infrequently observed in the caudal portion of intestine of fish in all groups. We could presume that Praziquantel was active at both dose rates and reduced the intensity of infection, but this was not statistically evaluated because of low initial prevalence of infection.

DISCUSSION

The evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts. Blood

Table (5): Show the relative level of protection against *Aeromonase hydrophila* vaccine

treatment	mortality	RLP
group A	5	4/9
group B	6	3/9
Control +ve	3	6/9
Control -ve	9	



Fig. (2): *Shape of tape worm isolated.*

indices are often subjected to variation depending upon the stress and environmental factors (Hlavova, 1993). More biochemical and toxicological researches have been conducted on mammals than on fish. However, not surprisingly, many biochemical similarities exist among vertebrate species (Hochachka and Mommsen, 1995). The main haematological response to administration of Praziquantel was a significant decrease in Hb content, PVC, RBC and antibody titer. This decline 48 h post-administration indicates a condition of light erythropenia and intralentic haemolysis. The decrease in these parameters may also be the result of poor mobilization of red blood cells from the spleen and other haematopoietic organs (Scott and

Rogers, 1981). Similar results were observed in fish exposed to pesticides (John, 2007). Buckley et al. (1976) reported that a prolonged reduction in Hb is deleterious to oxygen transport, and any blood dyscrasia and degeneration of RBC could be ascribed to a pathological condition in fish exposed to toxicants; however, the differences from control were found only after 48 h post-administration. We could assume from the results obtained 48 and 96 h after application, that decreases of these values are reversible.

The liver is an important organ for many metabolic processes and xenobiotic detoxification. Exposure to chemicals may lead to

their accumulation in the liver and/or may cause pathological alterations (Braunbeck, 1994). Moreover, hepatocyte injury leads to the release of tissue specific enzymes into the blood stream. The first sign of metabolic alteration could be a decrease in glucose due to decreased ability of the liver to ensure normoglycaemic level. High levels of ALT may indicate that hepatocytes have become inflamed or died (Hochachka and Mommsen, 1995). Any form of hepatocyte damage can result in elevated ALT, which is the most sensitive marker for liver cell damage. Praziquantel administration significantly increased plasma ALT in fish sampled after 48 and 96h compared with controls, suggesting plasmatic membrane damage. The activity of ALT did not differ from controls in any treated group. ALT is thought to be a predominantly non-mitochondrial enzyme, whereas approximately 80% of AST in hepatocytes appears to be located in mitochondria. When hepatocytes (as evidenced by plasmatic elevation of ALT), but not the mitochondrial membrane (no differences in AST), show damage, it has been postulated to be the

result of "mild" hepatocellular injury (Pincus and Schaffner, 1996). All methods of microcistin administration to fish also cause elevated ALT (Gupta and Guha, 2006). Vutukuru et al. (2007) recorded significant increases in ALT and AST activity in carp after exposure to chromium and arsenic. The effect of oral administration of Praziquantel, at different dose levels, on the activity of metabolizing hepatic enzymes was studied in rabbits by Kheir et al. (1995). Who found high doses of Praziquantel (1600 mg kg^{-1}) caused an increase of transaminase activity. This is in agreement with the results published by Bojorklund and Bylund (1987) who compared Praziquantel pharmacokinetics in mammals to those in fish and stated that the pharmacokinetic processes are slower in fish, and parasites are therefore subjected to the active drug for longer than in mammals.

In this study, other differences from controls were found in values of TP. Assessment of protein content can be considered a diagnostic tool to determine the physiological status of the cells (Manoj and Raghothaman, 1999). The slight

decline in TP may be related to ALT elevation as an initial sign of partial protein degradation, cell damage in plasma, and liver damage (Vutukuru et al. 2007). We do not know the time required for stabilisation of ALT activity.

In conclusion, results of the study have shown that Praziquantel treatment affects the physiological status of the important organs, as evidenced of differences in levels of transaminase activity and haematological indices in *O. niloticus*. However, the differences are not great and could presume their reversibility. No significant differences in parameters were found between the tested concentrations of Praziquantel. Further studies of the liver ultrastructure in fish may increase our understanding of pathological alterations in liver following praziquantel administration.

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تأثير البرازاكونتيل على بعض المقاييس الكيميائية ومقاييس الدم في البطي النيلي المصاب بالديدان الشريطية

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تهدف الدراسة الراهنة لدراسة أحد مضادات الديدان ممثلة في البرازاكونتيل على مقاييس الدم والمقاييس الكيميائية في البطي النيلي حيث تم تقسيم الأسماك إلى ثلاث مجموعات الأولى تعالج ب 40 مجم /كجم من وزن الجسم والثانية 60 مجم /كجم من وزن الجسم برازاكونتيل حيث تخلط جيدا مع العليقة .

تم فحص الأسماك كل 48 ساعة من إضافة الجرعة وقد أدى البرازاكونتيل إلى نقص ملحوظ في خلايا الدم الحمراء والهيموجلوبين خلايا الدم المثقلة وأيضا نقصان في نسبة البروتين الكلى من 48 إلى 96 ساعة عن المجموعة الضابطة. أيضا لوحظ زيادة في إنزيمات الكبد في نفس الفترة والذي يرجع إلى تكسير خلايا الكبد بتأثير البرازاكونتيل. بالإضافة إلى ذلك وجد نقصان في نشاط الليزوزيم والخلايا الأكلة وتبترت المضادات الحيوية ضد الايرومونات هيدروفيليا عن المجموعة الضابطة في خلال من 48 إلى 96 ساعة .