Study on Clinopathological and Biochemical Changes in Some Freshwater Fishes Infected With External Parasites and Subjected to Heavy Metals Pollution in Egypt

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Abstract: The present investigation was carried out to study the impact of external parasites and heavy metals pollution on some liver function tests of some freshwater fishes. 470 Fish species (330 *Oreochromis niloticus* and 140 *Clarias gariepinus)* were collected alive from three different ecosystems in Kafr-Elshiekh province, Egypt. The obtained results revealed that aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzymes activities as well as creatinine and urea values were elevated in the external parasites infected fish as well as in the fish exposed pollutants. While fishes exposed to both external parasites infection and heavy metal pollution led to more drastic increase in serum AST and ALT enzymes activities as well as creatinine and urea values. In addition; heavy metals pollution increased the susceptibility of fish to protozoa infection while decrease prevalence of monogenea and crustacean infection. On conclusion; infection with external parasites in fishes exposed to heavy metals had the highest effect on liver and kidney functions in the studied fishes.

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Introduction

Most of fish diseases might be occurred as a result of parasitic infection or environmental pollution (Hussain *et al.,* 2003) Knowledge of fish parasites is of particular interest in relation not only to fish health but also to understand ecological problems (Mahfous, 1997).

Aquatic pollution is still a problem in many freshwater and marine environments; it causes negative effects for the health of the respective organisms (Fent, 2007). The number of studies investigating effects of pollutants and concurrently occurring parasites is still relatively low (Sures, 2007). However the effect of environmental pollutants on fish parasites varies depending on the particular parasite and pollutant that interact (Lafferty and Kuris, *1999*). Pollutants may affect the immune system of the fish either directly or by change water quality; that in turn may reduce the fish immunity to parasites (Poulin, 1992) also, water pollution may accelerate the life cycle of the external parasites and promote their spread (Noor El-Din, 1997).

It is well known that certain blood parameters serve as reliable indicators of fish health as many parasites can live in a host, sometimes causing damage to it (Bond, 1979). Therefore, the changes associated with hematological parameters due to various parasites establish a database, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures. These measures are essential in fish farming and fish industry (Roberts, 1981).

Analysis of blood constituents is considered physiological indicators of the whole body and therefore they are important in diagnosis the structural and functional status of fish exposed to pollutants (Adhikari and Betal, 2004). In this respect; Ranzani-Paiva *et al*., (2000) demonstrated alterations in blood composition related to parasitism in fish from the Parana River, indicating that, determination of blood parameters of fishes is of great importance in evaluation of disturbance that caused by parasitism. Therefore, this study was aimed to investigate the impact of external parasites on some physiological parameters related to both liver and kidney functions of some freshwater fish *(Oreochromis niloticus* and *Clarias gariepinus)*, as well as to determine the relation between heavy metal pollution and the infection with external parasites.

2. Materials and Methods

Fish samples:

A total number of 470 (330 *Tilapia species* and 140 *Clarias gariepinus*) freshwater fish were collected a live from three different ecosystems at Kafr El-Shiekh governorate, North Egypt as follow. (River Nile Branch, Drainage canal and Fish farm) by the aid of fisherman and then transported a live to the laboratory where they examined immediately.

Parasitological examination

Parasitological examination was carried out for the detection and identification of the external parasites on the skin, gills and the accessory respiratory organs of the samples.

Collection and preparation of the detected ectoparasites

Monogenea:

Monogenea were collected under binocular dissecting microscopic by means of small pipette in small Petri-dish and cleared several times with water to remove the attached mucous and debris. The worms were then left in refrigerator at 4^{o}C till complete relaxation. Then, they were fixed in 5% formalin for permanent preparation, worms were washed carefully in water to get red of formalin traces and stained with Semichon's acetocarmine stain for about 5-10 minutes till reaching staining, the specimens were passed through ascending grades of ethyl alcohol (30, 50, 70, 90% and absolute) for dehydration. Then, cleared in clove oil, xylene and mounted in Canada balsam (Pritchard and kruse, 1982), while the unstained Monogeneas were mounted in glycerin jelly (Abdel-Hady, 1998).

Protozoa:

Some of the positive slides were stained according to Klein's dry silver impregnation method in which the slides were air –dried, covered with 2% aqueous solution of silver nitrate (Ag NO_3) for 8 minutes, rinse thoroughly in distilled water and exposed to UV light for 20-30 minutes or to direct sun light for 1-2 hr. The slides were allowed to dry and mounted with neutral Canada balsam. This method is indispensable technique for staining *Trichodina*. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain for 20-30 minutes to detect the other protozoa (Ali, 1992).

Crustacea:

The detected crustacean parasites were carefully collected by a fine brush and special needle, and transferred into Petri-dish for cleaning by using preserved and cleared in lacto phenol then mounting with polyvol (Raef *et al.*, 2000).

Heavy metals detection:

Three samples of water from the same sources of fish collection were taken in the fore mentioned flask one liter volume capacity after its rinsing several times with distilled water and sterilized in hot air oven at 180 ^{o}C/hour. The collected water sample bottles were labeled with the locality, date, and time of collection. Chemical examinations of these samples were done to estimate some heavy metals including (copper, zinc, iron, lead, cadmium, selenium, mercury, manganese and nickel) according to Chapman and pratt, (1978).

Blood samples

Fresh blood samples were collected without anticoagulant from the caudal artery. The needle is run, quite deep, as much as possible through a middle line just behind the anal fin in a dorso-cranial direction till striking the vertebrate. By drawing the needle gently backward, blood is usually sucked into the syringe.

The collected blood was centrifuged post collection at 3000 rpm for 10 minutes to separate serum for biochemical analysis.

Biochemical analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were determined according to Reitman and Frankel, (1975). Creatinine value was determined according to Rock *et al*., (1987). Urea concentration was measured according to Pathson and Nauch, (1977).

All these biochemical analyses were measured calorimetrically using spectrophotometer and purchased kits.

3. Results

The effect of heavy metals pollution on the prevalence of ectoparasites on examined fish spp. are shown in Table (1) which indicate that the percentage of ectoparsites infection was 71.8% from the examined *Tilapia spp* in the River Nile branch where the pollution of water with copper (Cu), nickel (Ni), cadmium (Cd), selenium (Se) and mercury (Hg) were higher than the other localities where the degree of pollutants were 2.190, 0.102, 0.260, 3.630 and 1.90 respectively, In this degree of pollution, the present study revealed that ectoprotozoa infection of *Tilapia* spp. were the highest percentage of infection where 65% of examined *Tilapia* fish were infected and it was followed by Monogenea and Crustaceans where they reached 23% and 13.7% respectively.

In Drainage canal, where the heavy metals pollution were lower than that in river Nile branch, 69% of examined *Tilapia* were infected with ectoparasites, where the protozoa parasite decreased than in river Nile branch 48%. While monogenea and Crustaceans increased (41%, 38% respectively).

In Fish farms; the percentage of infection reached 64.9% from the examined fishes. Parasitic protozoa decreased than in River Nile branch 46.9%, while monogenea and Crustaceans increased (38.0%, 34.5% respectively).

In addition; the effect of polluted water on the ectoparasitic infection of *Clarias gariepinus*, it was recorded that, in river Nile branch the fish infected with monogenea (28.7%) and protozoa (36.3%), while in Drainage canal (less polluted with heavy metals) monogenea increased 60% while parasitic protozoa decreased 20% than in River Nile branch. With no infection obtained with Crustacean parasites in the examined areas.

Table (1): Effect of heavy metals on parasitic infection among examined Fish spp. in different localities.

Locality	Parasitic infection	% of infection		Heavy metal pollutants in ppm								
				Zinc *1	Lead * _	Manganes *1.5	Copper *1	Nicke *_	Cadmium *0.01	Selenium *_	Mercury *_	Iron *1
		Tilapia spp	*C. gariepinus*									
Locality 1 (River Nile Branch)	Monogenea	23	28.7	0.2	0	0.08	2.19	0.102	0.26	3.63	1.9	0.12
	Protozoa	65	36.3									
	Crustacea	13.7	0									
	Total	71.8	53.7									
Locality 2 (Drainage canal)	Monogenea	41	60	0.06	0.5	0.12	0.07	0.102	0.04	0.26	0.25	0.06
	Protozoa	48	20									
	Crustacea	36	0									
	Total	69	65									
Locality 3 (Fish farm)	Monogenea	38	Not examined	0.07	0.5	0.42	0.11	0.068	0.07	0.52	0.15	0.81
	Protozoa	46.9										
	Crustacea	34.5										
	Total	64.6										

- * Permissible limits of trace elements detected in ppm. According to Egyptian Law
- No available guide line

As shown in Table (2); Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were higher in infected *O. niloticus* taken from River Nile branch than infected *O. niloticus* taken from other localities as they were (78.6 U/l), (115 U/l), (52 mg/dl) and (1.9 mg/dl) respectively while in non infected *O. niloticus* were (69 U/l), (101.5 U/l), (41 mg/dl) and (1.54 mg/d/) respectively. In Drainage canal, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (61.3 U/l), (96.7U/l), (37.3 mg/dl) and (1.4 mg/dl) respectively in the infected *O. niloticus* while in non infected were (51.5 U/l), (85U/l), (26.5 mg/dl) and (1.18 mg/dl) respectively. In Fish farm, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (67 U/l), (97.3 U /l), (36.3 mg/dl) and (1.6 mg/dl) respectively in the infected *O. niloticus* while in non-infected were (55 U/l), (81 U/l), (31.5 mg/dl) and (1.3 mg/dl) respectively.

Table (3) showed that in River Nile Branch, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were higher in infected *Clarias garipinus* (101.7 u/l), (177.7 u/l), (69 mg/dl) and (2.17 mg/dl) respectively . Than non infected *Clarias garipinus* as they were (85 u/l), (130 u/l), (50mg/dl) and (1.6 mg/d/) respectively. While in Drainage canal, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (73.3 u/l), (94u/l), (38.7 mg/dl) and (1.63 mg/dl) respectively in the infected *Clarias garipinus* while in non-infected were (54 u/l), (76u/l), (28.5 mg/dl) and (1. mg/dl) respectively.

Table (2): Liver and Kidney function tests of *O. niloticus* infected with ecto-parasites in different localities.

locality / Parameter	River Nile Branch		Drainage canal		Fish farm	
	Non-infected	infected	Non-infected	infected	Non-infected	infected
ALT (U/L)	69	78.6	51.5	61.3	55	67
AST (U/L)	101.5	115	85	96.7	81	97.3
Urea (Mg/dl)	41	52	26.5	37.3	31.5	36.3
Creatinene (Mg/dl)	1.54	1.9	1.18	1.4	1.3	1.6

Table (3): Mean liver and kidney function tests of *Clarias gariepinus* infected with ecto-parasites in different localities.

Locality / Parameter	River Nile Branch		Drainage canal	
	Non.infected	infected	Non.infected	infected
ALT (U/L)	85	101.7	54	73.3
AST (U/L)	13O	177.7	76	94
Urea (Mg/dl)	50	69	28.5	38.7
Creatinene (Mg/dl)	1.6	2.17	1.1	1.63

Table (4): Mean liver and kidney function tests of fish spp. infected with ectoparasites in different localities

Locality / Parameter	River Nile Branch				Drainage canal				Fish farm	
	O.niloticus		*C. gariepinus*		*O. niloticus*		*C. gariepinus*		*O. niloticus*	
	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non infected	Infected	Non infected	Infected
ALT (U/L)	69	78.6	85	101.7	51.5	61.3	54	73.3	55	67
AST (U/L)	101.5	115	13O	177.7	85	96.7	76	94	81	97.3
Urea (Mg/dl)	41	52	50	69	26.5	37.3	28.5	38.7	31.5	36.3
Creatinene (Mg/dl)	1.54	1.9	1.6	2.17	1.18	1.4	1.1	1.63	1.3	1.6

4. Discussion

A negative relationship was detected between heavy metals pollution and prevalence of monogenic infection in River Nile drainage canal branch as well as fish farm during this study. This result agreed with Blanar *et al.*, (2009) which may be attributed to the toxic effect of the heavy metal on the parasite itself Gheorghiu *et al.*, (2006).

The present study denoted that the incidence of external protozoa among examined fish was higher percentage in River Nile Branch which it is more polluted with heavy metals than other localities this may be attributed to that the heavy metals decrease the immune system of the exposed fish which become more susceptible to protozoa infection Khan and Thulin, (1991). The lowest rate of infection with parasitic crustacean was recorded in River Nile Branch, where the heavy metals pollution increased, this result agree with Galli *et al.*, (2001) who recorded that the distribution of *Lamproglena pulchella* was limited to the unpolluted and slightly polluted river sectors. This negative relation may be attributed to the toxic effect of the heavy metals on the crustaceans which may cut its life cycle Ruben *et al., (*2006).

The blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were elevated in the infected fish species *(Oreochromus niloticus* and *Clarias gariepinus*) with external parasites than the non infected fishes in different localities, this indicate that the external parasites stimulated the activities of ALT and AST enzymes as well as both Urea and Creatinine. This result was agreed with Younis, (1999) recorded that aspartate aminotransferase (AST), alanine aminotransferase (ALT) and urea showed significant increase in *O. niloticus* infected with external protozoa and monogenetic trematodes. Osman *et al.,* (2009) reported that blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were increased in Trichodina infected *Clarias gariepinus*.

Concerning the effect of parasitic infection on biochemical parameters of examined fish in the presence of heavy metal pollution, the present study indicated that the blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were more higher in both *Oreochromis niloticus* and *Clarias gariepinus* that examined from river Nile branch (more polluted locality with heavy metals) than infected fish spp. taken from drainage canal and fish farm (less polluted with heavy metals). This indicated that exposure of fish to parasitic infection in the presence of heavy metals is more powerful in stimulating the activities of ALT and AST enzymes (Adams, 2002). This may be due to hepatic cells injury or increased synthesis of the enzymes by the liver (Yang and Chen, 2003). The elevation in the urea level in the infected fish may be due to gill dysfunctions as the urea excreted mainly through the gills (Murray *et al.*, 1990). Also these findings may be attributed to the inflammatory reactions and intoxications produced by the parasite in the affected fish.

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