Biochemical and pathological studies on Trypanosomiasis among catfish "Clarias gariepenus"

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The present study was designed to investigate trypanosomiasis among one of native freshwater fish breed in Egypt namely catfish (*Clarias gariepenus*). Fifty fish were collected during summer season from the river Nile at Giza markets. The fish were examined for the presence of trypanosoma in the blood. Trypanosoma were detected in 10 (20%) of the collected fish. The main clinical signs of infected fish with trypanosoma were emaciation, dullness, respiratory distress, loss of escape reflex, mild ascitis and paleness of the gills. Post-mortem examination of infected fish revealed paleness of the internal organs (liver and kidneys) and slight congestion of spleen. Haematological examination of infected fish revealed significant decrease in erythrocytic count, haemoglobin and packed cell volume but significant increase in total leucocytic count accompanied with neutrophilia and eosinophilia. Serum biochemical analysis demonstrated a significant decrease in urea, total protein and albumin while a significant increase in AST, ALT, ALP, creatinine, glucose and γ-globulin were recorded. Microscopic examination of organ histopathological sections revealed cloudy swelling of hepatocytes with activation of kupffer cells, depletion of lymphocytes with thickening of tile trabeculae in spleen. While in kidney, necrobiotic changes of epithelial lining of renal tubules with vacuolation of glomeruli as well as hemorrhages were recorded.

Fish is considered as an important, valuable and highly nutritious source of animal protein. Several researches have been earned out on the effect of the parasitic diseases on wide range of fish species all over the world (Hemmingsen *et al.*, 2005) but little is known about blood parasitic diseases particularly in Egypt. Fish trypanosome causes changes ranged from changes in somatic indices and condition factors, anemia, and pale gills to general weakness, loss of escape reflex, emaciation and ascitis in infected fish (Kabata, 1985; Lom and Dykova, 1992; Laya, 1994 and Smit *et al.,* 2004).

Trypanosomiasis is associated with decreased erythrocytic count, serum proteins (Lom and Dykova, 1992 and Stoskopf, 1993). It is also associated with increased leukocytis count, alanine aminotransferase (ALT) enzyme, aspartate aminotransferase (AST) enzymes and blood glucose level (Lom and Dykova, 1992 and Stoskopf, 1993). The lowered serum proteins are attributed to hepatic dysfunction and degradation of proteins by proteolytic enzymes (Stoskopf, 1993).

Haemoflagellate parasitic disease induces physicochemical character including lysozyme, haemolysin reactive protein, lectins and the epidermal migration of inflammatory cells (Jones, 2001). In addition infection with blood flagellate parasites inside the cell induces apoptosis (Saeij *et al*., 2003). The relationship between heavy experimental infection of *Trypanosoma danuilewshyi* and extensive damage of haemopiotic tissue has been proved (Dykova and Lom, 1979 and Woo, 2004). Histopathological changes regarding mainly the haemopiotic organs in fish infected with trypanosomes as well as heamobiotic damage and death were recorded (Lom and Dykova, 1992 and Noya, 1996).

This work aimed to spot a light on prevalence of trypanosomiasis in Egyptian native freshwater fish (*Clarias gariepenus*) as well as its effect on fish haemopiotic system, liver and kidney functions as well as protein electrophoretic pattern.

Material and Methods

Fish. A total number of 50 freshwater fish (*Clarias gariepenus*) were collected from river Nile at Giza market during summer season with average body weight 150 ± 10 g. These fish were transported alive to laboratory of Animal

Health Research Institute at Dokki in prepared large plastic bags and kept in prepared full glass.

Clinical examination. The fish were fed commercial pellets and they were subjected to clinical and post-mortem examinations according to the method described by (Lucky, 1977).

Parasitological examination. Fresh blood films stained with diluted Giemsa stain and examined microscopically for detecting trypanosomes according to Kabata (1985).

Blood collection. Blood samples were collected from caudal vein, blood samples divided into two portions, the first portion was sampled with heparin as anticoagulant for haematological study and the other left without anticoagulant and kept to clot. Serum was separated and was kept at -20ºC until processing for biochemical analysis.

Haematological examination. Estimation of erythrocytic count, haemoglobin (Hb), packed cell volume (PCV) and total as well as differential leucocytic counts were performed according to method described by (Lucky, 1977).

Serum analysis. Serum samples were colorimetrically analyzed for aspartate amino-transferase "AST", alanine aminotransferase "ALT" (Reitman and Frankel, 1957), ALP (Roy, 1970), total protein (Peters, 1968), protein fractionation using polyacrylamide gel electrophoresis (Davis, 1964 and Ornstein, 1946); creatinine (Faulkner and King, 1976); urea (Coulombe and Favreau, 1963) and glucose (Trinder, 1969).

Histopathological examination. For histopath-ological examination tissue samples were taken from liver, spleen, kidney and muscles. The samples were fixed in 10% neutral buffer formalin, processed by paraffin embedding method, sectioned at 4-5 µm and stained with hematoxylin and eosin (Bancroft *et al*., 1994).

Statistical analysis. The obtained data were statistically analyzed and tested for significance according to (Petrie and Waston, 1999).

Results and Discussion

Parasitological examination for trypanosoma in collected fish (50) revealed that 10 fish (20%) were trypanosome positive and concurrently were tested for all parameters in comparison to apparently healthy 10 fish.

Examination of stained blood film reveled presence of trypanosomas between blood cells as seen in Fig. (1).

The clinical picture showed that infected fish with trypanosomes were emaciated, dull and suffered from respiratory distress, loss of escape reflex, mild ascitis and paleness of the skin and gills. The observed clinical signs were nearly similar to those observed by (Lom and Dykova, 1992; Laya, 1994 and Awad, 1997).

Post-mortem examination revealed paleness of the internal organs (liver, kidney) and slight congestion of spleen as result of dysfunction of the haemopiotic organs especially the kidney. This observation was nearly similar to those obtained by (Brown and Gratzek, 1980; Awad, 1997 and Smit *et al*., 2004). The paleness of internal organs of infested fish was due to hemolytic anaemia that is induced by haemolysins secreted by live trypanosoma (Lom and Dykova, 1992). This results in blood insufficiency and difficult respiratory with subsequent compensatory spleenomegaly. These results were nearly similar to that obtained by (Awad, 1997 and Smit *et al*., 2004).

Concerning the haematological picture, the trypanosoma infection resulted in significant decrease ($P<0.001$) in RBCs count, Hb content, ($P< 0.01$) PCV % and lymphocyte as well as a significant increase in total W.B.Cs. count, neutrophil and eosinophils (Table, 1). These obtained results were agreeing with that recorded by (Khan *et al*., 1980; Sahlab *et al*., 1990; Lom and Dykova, 1992 and Awad, 1997) and confirmed histopathologically by the presence of hemorrhage in the haemopiotic organs as well as haemosidrosis (Fig., 4).

The marked increase in the total leucocytic count may be due to acute stage of haemolytic anaemia, which is always associated with leucocytosis (Duncan and Prasse, 1989). Regarding the eosinophilia produced by trypanosomes it may be due to eosniophilic chemotactic factor secreted by such protozea. The lymphopenia may be either to low oxygen tension as respiratory distress and reflected negatively effect on the process of lymphopiosis (Robert, 1989). Lymphatic depletion of the spleen is shown in (Fig., 3).

The effects of trypanosoma on serum constituents of infested fish C. gariepenus were shown a significant decrease ($P < 0.01$) in serum urea, ($P<0.001$) in total protein and albumin. On the other hand, there was a significant increase ($P<0.001$) in serum enzymatic activities of AST, ALT, ALP, creatinine, and glucose and ($P<0.01$) in gamma globulins. However both alpha and

beta globulins did not show any significant alteration (Tables, 2, 3).

The observed decrease in serum urea level was nearly similar to that recorded by (Tandron and Chandra, 1973) who attributed this decrease in serum urea level to probable inhibition in urea synthesis as a result of hepatocellular damage. This observation was proved histopathologically by changes, which observed in hepatocytes (Fig., 2).

The recorded increase in serum enzymatic activities of AST, ALT and ALP, agrees with that was reported by (Stoskoph, 1993 and Awad, 1997). This could be regarded to the observed histopathological hepatocellular damage (Fig., 2). The observed elevation in serum creatinine level could be attributed to the obtained renal dysfunction. The present histopathological observation augmented this conclusion by reported hepato-renal damage (Fig., 2 and 4).
The observed hyperglycemia nearly accordance with that reported by Joshi (1982). and could be resulted from stress action of corticosteroids on carbohydrate metabolism that results in the process of gluconeogenesis (Duncan and Prasse, 1989).

In our work, the electrophoretic pattern of serum protein and its fractions revealed a significant decrease ($P<0.001$) in albumin. This is accompanied by a significant elevation ($P<0.01$) in gamma globulin. This result is nearly similar to that obtained by Khan *et al*. (1980). Observed drop in serum total protein may be attributed to hepatocellualr damage as well as increase capillary permeability for plasma protein and degradation of protein by proteolytic enzyme released from endothelial cells destroyed by causative agents (Stoskoph, 1993).

Histopathological findings, in liver, showed vacuolar degeneration, hepatic necrosis and dilatation of blood vessels (Fig., 2). Spleen showed depletion of lymphocytes with thickening of trabecule (Fig., 3). Kidney of fish showed necrosis, degeneration and haemorrhage (Fig., 4). This observation nearly agreed with that reported by (Dykova and Lom, 1979; Lom and Dykova, 1992 and Noya, 1996) who attributed these changes to the pathogenic effect of trypansomes.

In conclusion, this study proved that trypanosomiasis induced extensive damage of haemopiotic tissue of catfish "*Clarias gariepenus*" that is reflected reflect on health status of the fish.

Table (1): Eythrocytic and leycocytic cell counts as well as differential leucocytic count in *Clarias gariepenus* fish infected with trypanosoma.

	Control	Infected
RBCs (x 10^6/mm^3)	2.64 ± 0.13	1.81 ± 0.12**
Hb (g/dl)	15.45 ± 0.54	12.11 ± 0.46**
PCV (%)	28.1 ± 1.62	22.11 ± 0.95*
WBCs (x 10^3/mm^3)	15.44 ± 0.37	18.39 ± 0.49**
Neutrophil (x 10^3/mm^3)	2.16 ± 0.09	4.68 ± 0.11**
Eosinophil (x 10^3/mm^3)	0.69 ± 0.16	1.79 ± 0.19**
Basophil (x 10^3/mm^3)	0.07 ± 0.01	0.09 ± 0.02
Lymphocyte (x 10^3/mm^3)	12.36 ± 0.14	11.72 ± 0.12*
Monocyte (x 10^3/mm^3)	0.15 ± 0.07	0.11 ± 0.08

Table (2): Serum enzymatic activities (U/L) as well as creatinine, urea and glucose (mg/dl) in *Clarias gariepenus* fish infected with trypanosoma.

	Control	Infected
AST	29.23 ± 0.73	47.9 ± 1.18**
ALT	21.84 ± 0.57	39.89 ± 1.01**
AST/ALT	1.338 ± 0.018	1.200 ± 0.028**
ALP	82.44 ± 1.99	156.49 ± 2.65**
Creatinine	0.54 ± 0.019	0.71 ± 0.03**
Urea	2.71 ± 0.11	2.11 ± 0.12*
Glucose	55.4 ± 1.35	66.4 ± 2.15**

n =10 * Significant at $P < 0.01$ ** Significant at $P < 0.001$

Table (3): Showing serum total protein level and its fractions (g/dl) in *Clarias gariepenus* fish infected with trypanosoma.

	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)		
			α	β	γ
Control	4.98 ± 0.14	1.95 ± 0.12	1.86 ± 0.082	0.68 ± 0.023	0.49 ± 0.037
Infested fish	4.23 ± 0.13**	1.11 ± 0.09*	1.78 ± 0.051	0.67 ± 0.042	0.66 ± 0.031*

N = 10
* Significant at P < 0.01
** Significant at P < 0.001

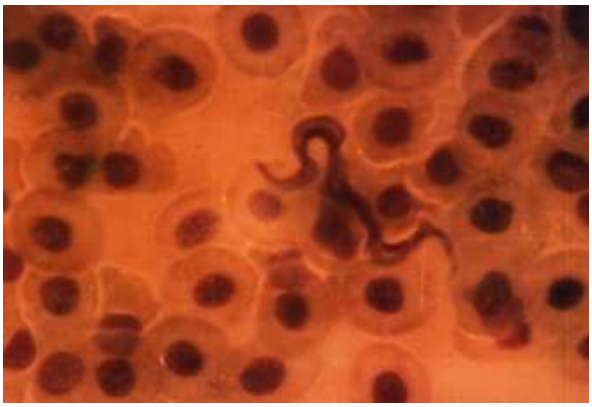

Fig. (1): Blood film of *Clarias gariepenus* fish showing trypanosome between blood cells (Giemsa stain x 100).

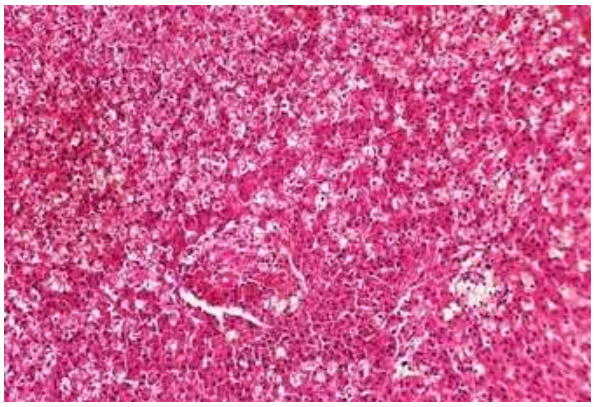

Fig. (2): Liver of *Clarias gariepenus* showing vacuolar degeneration, hepatic necrosis and dilatation of blood vessels (H & E x 400).

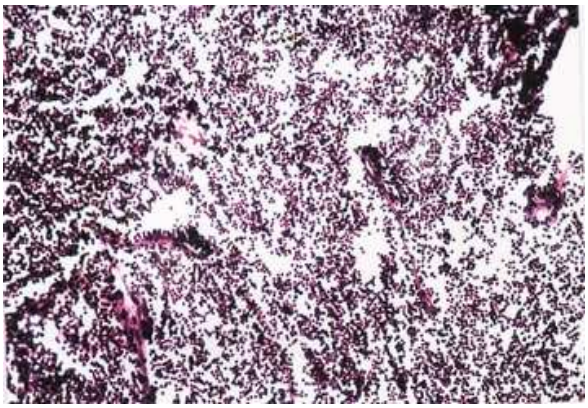

Fig. (3): Spleen of *Clarias gariepenus* fish showing depletion of lymphocytes with thickening of trabecule (H & E x 400).

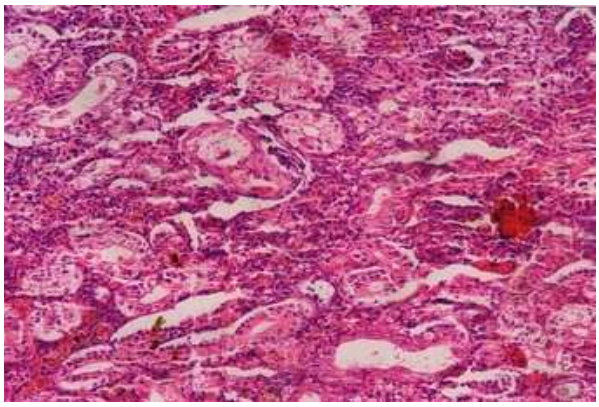

Fig. (4): Kidney of *Clarias gariepenus* fish showing necrosis, degeneration and haemorrhage (H & E x 400).

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