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Research Article
Trypanosoma Infection in African Sharptooth Catfish *Clarias gariepinus* with Special Reference to Control

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Abstract

Background and Objective: Trypanosoma infection in fish considered as the extreme important internal disease affect fishes. The present study focused on trials for treatment of trypanosome infection in African sharptooth catfish with histopathological study of the naturally infested fish. **Materials and Methods:** A total number of 200 alive catfish with body weight 120±10 g were obtained from private fish farm. The fish subjected to parasitological investigation for trypanosomes. For treatment trials, a total number of 120 naturally infected catfish were divided into 4 groups each 10 were used for treatment trial with Benznidazol I/M (5 mg kg^{-1} b.wt.), bath with *Aloe vera* leaves aqueous extract (50 and 100 mg L^{-1} for 120 min). **Results:** The main clinical and postmortem lesions were paleness of the body surface, eroded fins, gulping the atmospheric air and surfacing. Trypanosoma infection caused significant decrease in red blood counts, haemoglobin and PCV. Serum total protein, albumin, globulin concentrations, albumin/globulin (A/G) ratio and cholesterol concentration. Histopathological studies displayed necrotic and inflammatory reaction in gills and skin, *C. gariepinus*, *Oreochromis niloticus*, gold fish *Carassius auratus* and male white mice subjected to experimental infection with trypanosome. The prevalence of trypanosome infection in catfish was 53%. For treatment trials, the results revealed that 100 mg L^{-1} for 120 min was the best treatment for trypanosomiasis. **Conclusion:** It was concluded that the best treatment for trypanosoma infection in sharptooth, *C. gariepius* was, bath treatment with *Aloe vera* ethanol extract (100 mg L^{-1} for 120 min).

Key words: Trypanosomiasis, *Clarias gariepinus*, haematological, emaciation, histopathological changes, treatment, benznidazole, *Aloe vera*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

With the worldwide fish production and intensive cultivation system, fish are subjected to a wide spectrum of diseases which lead to great losses and decrease of fish production.

Protozoan parasites was known for many years to affect several groups of fishes and cause great economical damage to their host fish. In many instances individuals of protozoan parasites causes the secondary infections[1]. Blood parasitic diseases of fishes were prevalent in Egypt. May be due to the long periods of optimum warm water and consequent, abundance of natural food as well as the availability of the intermediate hosts (Cyclops, mollusca and leeches)[2,3]. Which affects fishes directly causing high morbidity and mortality by lowering the body gain and results in high economical losses[4]. Trypanosomiasis of fish caused by genus *Trypanosoma* which considered the most important economic internal disease, affecting freshwater fish inherited by crustacean parasites[5,6]. Such infection makes infected fish suffering from anemia with dulla appearance, secondary infection and cannibalism[7,8,9,2].

Example of phytotherapy, widely used in herbal medicine, is the *Aloe vera* plant, known as "babosa". It has been used over the years to treat various diseases and have been referred to as the "miracle" plant. It has been suggested that the extract of the plant promotes healing of wounds through synergistic action of many substances and some specially prepared *A. vera* such as anti-inflammation, anti-cancer, antidiabetes, macrophage stimulation, combat intestinal infections and urinary infection, as an analgesic Reynolds and Dweck[10].

Metronidazole is a product of synthetic chemistry. Azomycin, produced from the extract of soil streptomyces, was the first 2-nitroimidazole to have activity against protozoans, specifically Trichomonas. Benznidazole is another 2-nitroimidazole-based drug and have therapeutic use in chagas disease due to trypanosomiasis Otigbuo and Woo[11] and Anand and Wakode[12].

There is no available literatures investigate the control of trypanosome infection in fish so that, the present investigation was focused on to make some trials for treatment of trypanosome infection in African sharptooth catfish using Benznidazole and *Aloe vera* (babosa) aqueous extract with hematological, biochemical and histopathological examination of the naturally infested catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Sample collection: A total number of 200 alive catfish (*Clarias gariepinus*) with an average body weight of 120±10 g were randomly collected from private fish farm. Fish transferred to department of Hydrobiology, National Research Centre, Egypt, from March, 2017 to April, 2018. Fish was kept in fully prepared glass aquaria (1.5×2.0×1.5 cm) at 20°C for 2 weeks and subjected to parasitological examination for the trypanosoma species. Then subjected to experimental infection. All fish were fed with commercial diet 2.5% body weight twice daily. Blood was collected from the caudal vein with 1 mL heparinized syringe, examined and the parasitemia was estimated from wet blood preparation[13]. The trypanosomes infected fish were then separated from the trypanosomes free fish. The fish were then subjected to clinical, postmortem, cross infection and histopathological examinations.

Clinical and postmortem examinations: The infected catfish were subjected to the clinical as well as postmortem examinations using methods described by Lucky[13] for determination any external and internal abnormalities on the external body surface and internal organs.

Parasitological examination: Fresh blood samples were collected from the caudal veins according to Lied *et al.*[14]. Blood films were prepared, air dried, fixed with methanol and stained with freshly prepared and diluted Giemsa stain. The stained blood films were examined with lens ×400 or oil immersion lens of light compound microscope to detect the presence of trypanosomes according to Kabata[15].

Haematological and biochemical studies: Fresh blood samples were collected from 10 trypanosoma infected catfish and 10 trypanosoma free catfish. Collected blood were divided into two portions. Blood without anti-coagulant for serum preparation for the biochemical examination. Total protein level in serum was determined according to Cannon *et al.*[16]. Serum albumin concentration was measured as described by Gustafsson[17]. Blood serum globulin was calculated by subtracting the concentration of albumin from that of the total protein and albumin/globulin ratio (A/G ratio) was calculated by dividing albumin concentration over that of globulin Coles[18]. Urea concentration was measured according to Pathson and Nauch[19]. Creatinine level was determined after Rock *et al.*[20]. Uric acid concentration was determined

according to Schultz[21]. Cholesterol content was measured according to Stein[22]. Activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel[23]. Blood with anti-coagulant (EDTA) for haematological parameters determination.

Experimental infection: From 5 naturally infected catfish *C. gariepinus*, blood withdrawn with heparinized syringe from the caudal blood vessels, pooled and injected I/P into 5 *C. gariepinus* and injected I/M into 5 another *C. gariepinus*, injected also into 10 *Oreochromis niloticus* (5 fish I/P and 5 fish I/M) and injected also into 10 gold fish *Carassius auratus* (5 fish I/P and 5 fish I/M). All injected fishes were examined for trypanosomiasis infection 5 days intervals, making samples from blood film, dried and fixed with methanol and stained with freshly diluted Giemsa stain and examined with oil immersion light microscope.

Drugs and plants used in treatment of trypanosomiasis

Preparation of plant extracts: *Aloe vera* plant purchased from National Research Center, The leaves were washed thoroughly in running tap water to remove sand and debris. Thereafter, they were dried by spreading under the sun for 3 days and finally in a hot air oven at 60°C for 8 h. *Aloe vera* pulp was also obtained after cutting the plant longitudinally into two. The dried leaves were crushed to powder in a mortar and pestle and subjected to Soxhlet extraction with an aqueous extracting solvent. The solvent was exhausted from the extract with the help of a rotary evaporator. The phytochemistry analysis of the plant extracts was carried out as described by Odebiyi and Sofowora[6] to test for the presence of tannins, resins, glycosides, flavonoids, alkaloids and saponins. The extract was stored in a refrigerator until required for use[24].

Experimental design for treatment of naturally infected catfish: A total number of 120 *Clarias gariepinus* naturally infected fish with trypanosome mukasi divided into 4 groups each 10 fish with 3 replicate, first group was injected I/M with Benznidazole with a dose 5 mg kg^{-1} b.wt., of fish and 2nd group subjected to bath treatment with *Aloe vera* leaves an aqueous extract bath (50 mg L^{-1} for 120 min)[24] and 3rd group was subjected to *Aloe vera* leaves an aqueous extract bath (100 mg L^{-1} for 120 min)[24] and 4th group was set as a control group with no treatment. All groups were subjected for examination 5 days intervals for presence of trypanosome in blood. Heparinized blood was withdrawn from treated and untreated fish.

Histopathological examination: Infected fish with trypanosome as well as treated fish were subjected to histopathological examination. Tissue specimens were rapidly fixed in Davidson's fixative for 24 h then transferred to 70% ethanol till processing proceeds. The fixed specimens were processed through the conventional paraffin embedding technique (dehydration through ascending grades of ethanol, clearing in xylene and embedding in paraffin wax at 60°C). Paraffin blocks were prepared and cutting 3 µm-thick tissue sections by using microtome (Leica 2155). Then the slides were stained with H and E stain then examined by light microscopy according to Bancroft and Gamble[25].

Statistical analysis: Data were presented as mean±standard error (SE) and the significance of differences was estimated using Student's t-test as described by Snedecor[26].

RESULTS

Clinical picture and postmortem lesions: The present study revealed that the examined catfish naturally infected with trypanosoma displayed paleness of the body surface, emaciation with eroded fins Fig. 1a gulping the atmospheric air with dullness, exhausted and dull appearance with pale gills and dendritic organ Fig. 1b. Internal examination displayed friable spleen, watery blood with paleness of the internal organs.

Parasitological investigation: External features of protozoan trypanosoma of the present study were extremely meet with *Trypanosoma mukasai* (Fig. 2).

Prevalence of trypanosome infection in infected catfish: Prevalence of trypanosomiasis in infected catfish was about 53%. From out 200 cultured catfish *Clarias gariepinus*; 106 fish was infected with *Trypanosoma mukasai* with percentage of 53%.

Hematological and biochemical results in serum: As shown in Table 1, red blood cells, haemoglobin and PCV were significantly decreased. Serum total protein, albumin, globulin concentrations, albumin/globulin (A/G) ratio and cholesterol concentration were significantly decreased in trypanosoma diseased catfish in comparing with non infected catfish. Aspartate aminotransferase and alanine aminotransferase enzymes activities in the serum of diseased catfish were significantly increased in comparison with the non diseased catfish. However the urea, creatinine and uric acid

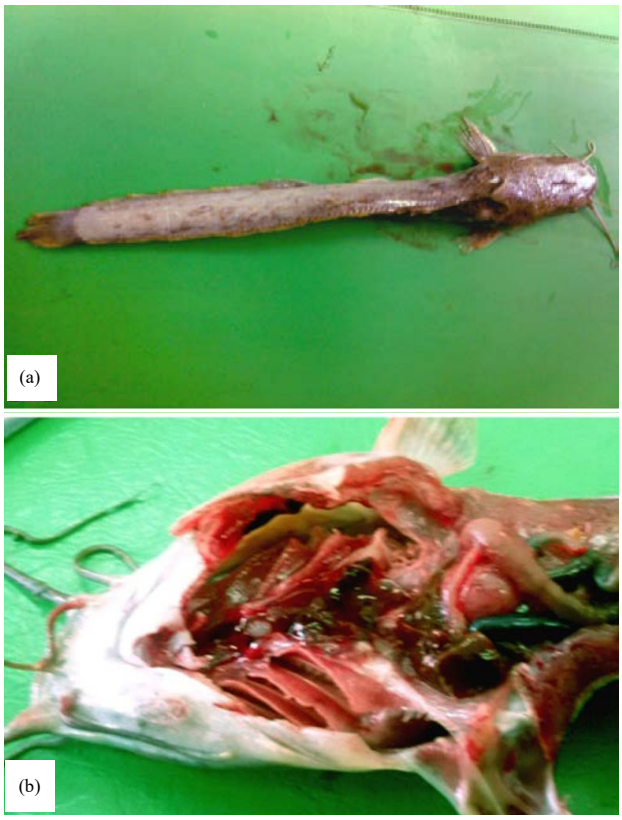

Fig. 1(a-b): Catfish *Clarias gariepinus* revealed, (a) Emaciation, eroded fins with pallness of body surface and (b) Pale gills and dendritic organ with congestion

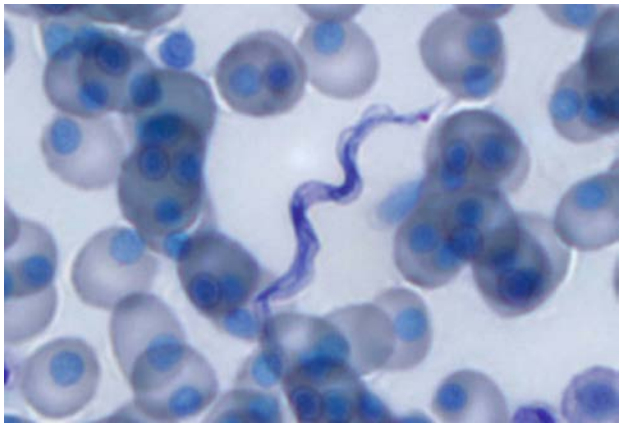

Fig. 2: Light micrograph of giemsa stained *Trypanosoma mukasai* in blood film from naturally infected *C. gariepinus*

concentrations in trypanosoma infected catfish were not significantly changed in comparison the trypanosoma free catfish.

Table 1: Some hematological and serum biochemical parameters in infected catfish with trypanosoma

Parameters	Groups: Trypanosoma free fish	Groups: Trypanosoma infected fish
RBCs (10^6 µL^{-1})	3.42±0.14	1.44±0.12*
Hb (g dL^{-1})	9.91±0.41	7.59±0.55*
PCV (%)	30.10±1.15	25.23±1.44*
Total protein (g dL^{-1})	6.52±0.22	4.95±0.12*
Albumin (g dL^{-1})	3.40±0.08	2.70±0.03*
Globulin (g dL^{-1})	5.22±0.20	3.37±0.10*
A/G ratio	1.59±0.02	0.37±0.02*
Cholesterol (g dL^{-1})	198.20±5.15	119.60±3.02*
Urea (g dL^{-1})	26.54±1.85	30.33±1.73
Creatinine (g dL^{-1})	0.91±0.02	0.95±0.02
Uric acid (g dL^{-1})	5.10±0.17	5.55±0.20
AST (U dL^{-1})	57.90±2.16	996.41±5.67*
ALT (U dL^{-1})	38.85±1.80	88.68±3.70*

Each value represents Mean±SE, n = 10, *Significant difference by student t-test at $p \leq 0.01$

Table 2: Cross infection from *C. gariepinus* to other species

Received species	I/P injection	I/M injection	Subcutaneous
Claris gariepinus	+ve	+ve	-ve
Oreochromus niloticus	+ve	+ve	-ve
Goldfish *Carassius auratus*	+ve	-ve	-ve

Experimental infection (cross infection): It was evident that the transmission of *Trypanosoma mukasai* from infected *C. gariepinus* to free from trypanosome was succeeded through two routes of infection I/M and I/P also, *O. niloticus* showed +ve infection through two routes I/P and I/M and -ve trough subcutaneous while, goldfish showed only route of infection I/P and another was -ve (Table 2).

Treatment of trypanosomiasis in naturally infected *C. gariepinus*: It was evident from the results that the effect of treatment was determined throughout lower number of died fish with highest rate of treated fish. From the results it was clear that the highest rate of treatment was for *Aloe vera* extract in the 3rd group for 9 fish 90% treatment followed by 1st group 8 fish treated with percentage 80% 7 fish survival followed by 2nd group 4 treated fish with percentage 40% and 9 survival fish, while the little rate of treatment was for 4th group 0 treatment and 5% survival (Table 3).

Histopathological examination: The histopathological changes resulted from trypanosoma infection displayed, kidneys showing severe degenerative changes in the epithelium of tubules, lining the glomerular epithelium and interstitial hemopoietic tissues, congestion in renal blood vessels revealed per glomerular and per tubular edema (H and E, ×400) (Fig. 3a). Liver displayed vacuolation and degenerative changes in the hepatic cells, congestion in the

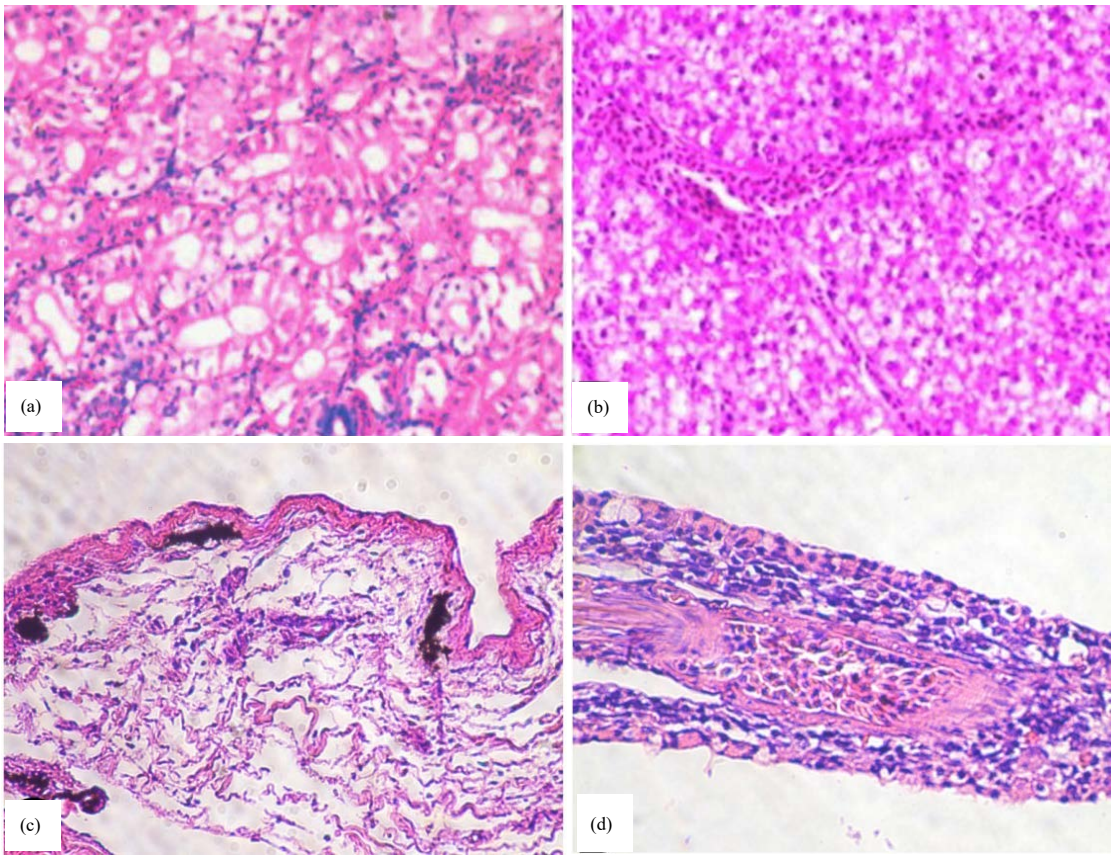

Fig. 3(a-d): Catfish infected with trypanosome, (a) Kidneys suffered from severe degenerative and necrotic changes in the tubular epithelium, endothelial lining the glomerular collection and in the interstitial hemopoietic tissues, congestion in renal blood vessels and per tubular and per glomerular edema (H and E, ×400), (b) Liver suffered from vacuolation and necrotic changes in the liver cells, congestion in the blood vessels, hyperplasia in the wall of blood vessels and infiltration of inflammatory cells and fibrous tissues between the hepatic parenchyma (H and E, ×400), (c) Skin trypanosoma infection revealed necrotic changes in the epidermal layer, sub epidermal edema, necrosis and infiltration of inflammatory cells in between the muscle bundles and (d) Gills showed severe vacuolar degeneration in the epithelial lining the secondary lamellae associated with hyperplasia (H and E, ×200). Gills showing congestion of the blood vessels, hyperplasia in the respiratory epithelium lead to adhesion between the secondary lamellae (H and E, ×400)

Table 3: Treatment effeciency in trypanosomiasis in experimentally infected *C. gariepinus*

Groups	No. of fishes	Drugs	Dose	Treated	Survival	Treated fish (%)
1	10	Benznidazole	5 mg kg^{-1} b.wt.	8*	7	80
2	10	Aloe vera	50 mg L^{-1} for 120 min	4	9	40
3	10	Aloe vera	100 mg L^{-1} for 120 min	9*	9	90
4	10	No treatment	Control	0	5	0

Each value represents Mean±SE, n = 10, *Significant difference by student t-test at $p \leq 0.01$

blood vessels, hyperplasia in the wall of blood vessels and infiltration of inflammatory cells and fibrous tissues between the hepatic parenchyma (H and E, ×400) (Fig. 3b). C-skin trypanosoma infection displayed degenerative changes in the epidermal layer, epidermal edema, zenkers necrosis and infiltration of chronic inflammatory cells in between the muscle bundles (Fig. 3c, d). Gills displayed severe degeneration and necrosis in the epithelial lining the secondary lamellae accompanied with hyperplasia (H and E, ×200).

DISCUSSION

The present study was aimed to investigate trials for treatment of trypanosoma infection in African catfish using Benznidazole and *Aloe vera* (babosa) ethanolic extract with histopathological examination of the naturally diseased catfish, *Clarias gariepinus*.

Concerning the clinical sings and postmortem lesions, present study revealed that the examined catfish that infected with trypanosome revealed paleness of the body surface, slim body and eroded fins, gulping the air with lethargy, or congestion of gills with dendritic organ. Internal inspection revealed spleen enlargement, watery blood. These clinical signs were nearly the same that obtained by Mariam[27], Essam and El-Khateib[28], El-Khatib and Elias[29], Adawy and Deeb[30]. Trypanosoma infection produce haemolysins that lyse the RBCs. Anemia caused was by hemolysin secreted by parasite. Regarding the prevalence present investigation displayed that the prevalence of trypanosomiasis in catfish *Clarias gariepinus* was about 53% from out 200 cultured catfish *Clarias gariepinus*; 106 fish infected with *Trypanosome mukasai* with percentage 53%. The results nearly agreed with that obtained by Overath *et al*.[8], Ahmed[31] and Kidchakan[32]. These phenomena may be due to the fact that the *C. gariepinus* fish were are non scaly fish and these fish were active and live at the bottom of the pond as it is bottom feeder where leeches (blood sucking vector) were natural inhabitant.

In regard with the haematological and biochemical findings in the serum of trypanosoma infected catfish. It was found that the RBCs count, Hb and PCV were significantly decreased, total serum protein, serum albumin and globulin as well as A/G ratio were significantly decreased comparing with the non infected fish. Trypanosoma infection in fish causes generalized edema and haemodilution Osman *et al*.[33] and Gupta[34]. So, the decrease in the serum total protein, albumin and globulin in the present study may be due to infection. Low A/G ratio may indicate inflammation and liver aliment Jacobs *et al*.[35]. Cholesterol level was significantly decreased in trypanosoma infected catfish. The present result nearly agreed with that of Tandon and Chandra[36] in trypanosomes infected *Clarias batrachus*, *Wallagoattu*, spiny eel *Mastocem- belus armatus* and the carp Cirrhina mrigala. The hypocholesterolemia may be also due to depressed de novo synthesis Ferrando and Andreu-Moliner[37] and Osman *et al*.[2]. AST and ALT activities were significantly elevated in trypanosoma infected catfish in comparison with the trypanosome non infected fish as explained by Yang and Chen[38]. On the other hand, the levels of urea, creatinine and uric acid were not changed in *C. gariepinus* infected with trypanosoma in comparison with the healthy fish.

Concerning the experimental infection of *Trypanosome mukasai* from catfish *C. gariepinus* to catfish *Clarias gariepinus*, *O. niloticus* and Goldfish *Carassius auratus*. The present study revealed that the transmission of trypanosoma mukasai from infected *C. gariepinus* to free *C. gariepinus* from trypanosome was succeeded through two routes of injection I/P and I/M also, *O. niloticus* showed +ve infection through two routes I/P and I/M and -ve trough subcutaneous while, *Carassius auratus* showed only one route of infection I/P. The results agreed with the results of Woo and Black[39] who reported that trypanosome is not host specific however trypanosoma danilewski Laverne and Mesnil, 1904 causes mortality in experimentally infected goldfish *Carassius auratus*[40,41].

Regarding trials for control of trypanosome infection in catfish, present study displayed that the effect of treatment was determined throughout lower number of died fish with high test rate of treated fish. From the results it was clear that the highest rate of treatment was for *Aloe vera.* In the 3rd group treatment for 9 fish 90% treatment followed by 1st group 8 fish treated with percentage 80% 7 fish survival followed by 2nd group 4 treated fish with percentage 40% and 9 survival fish, while the lowest level of treatment was for 4th group 0 treatment and 5% survival.

The obtained results was supported by Several modern studies which have also revealed that the extract of the plant promotes healing of diseases through the synergistic interaction of many substances, specially prepared *Aloe vera* extracts, possess some biological activities such as anti-inflammation, anti-cancer, anti-diabetes, decrease gastrointestinal infections and stop urinary infections, as an analgesic[24].

Concerning the histopathological changes of naturally infested catfish in the present study revealed degenerative, necrotic and inflammatory changes in all internal organs, skin and gills these results confirmed by Bunnajirakul *et al*.[42], Supamattaya *et al*.[43].

Treatment of trypanosomiasis in catfish must be concentrated in the future on the extracts of medicinal plants because it is safe and economic without any side effects on fish and environment.

CONCLUSION

From the present study, it was concluded that, trypanosome infect catfish and most freshwater fishes and it is not host-specific also concluded that the practical and economic drug of choice for trypanosomiasis treatment in African catfish *C. gariepinus* was the ethanolic extract of leaves of *Aloe vera* as a bath100 mg L^{-1} for 120 min.

SIGNIFICANCE STATEMENT

This study discovered the pathogenesis of trypanosomiasis in sharptooth African catfish and discovered also applicable medical treatment for trypanosomiasis in African catfish. Treatment was the ethanolic extract of leaves of *Aloe vera* as a bath100 mg L^{-1} for 120 min. It is safe and economic without any side effects on fish and environment. This study will help the researcher to uncover the critical areas of treatment parasitic protozoal diseases affect catfish that many researchers were not able to explore.

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