Histopathological changes in diseased catfish (Clarias gariepinus) and treated by ciprofloxacin and clove extracts.

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Abstract

Bacteria pathogens may cause a serious loss in aquaculture and also health hazards to humans. On the basis of biochemical characteristics and API-20E system, the highest prevailing isolate was identified as *Aeromonas sobria.* Infection with *A. sobria* in African catfish (*Clarias gariepinus*) induced focal hemorrhage at the end of gill operculum, generalized edema and ulcers on skin surface. Internal signs of infection include hyperemia of the hepatic artery, coagulative necrosis in the liver and hydropic degeneration of renal tubules according to the histopathological examination. *In vivo* the combination of ciprofloxacin and clove extract was the choice as alternative bacterial therapeutic agents in infected *C. gariepinus* with *A. sobria*. Moreover histopathological changes have is the base for the evaluation of fish health.

Keywords: *Aeromonas sobria*, Catfish, Ciprofloxacin, Clove.

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Introduction

The most common pathogen in channel catfish is *Aeromonas* spp., which is the causative organism of motile aeromonas septicemia (MAS) and can infect multiple fish species including tilapia, catfish, gold fish and common carp [1]. MAS is one of the cultured warm-water fish diseases in fresh water environments [2]. Nam and Joh (2007) noticed that the dominant strain at the farm during all four seasons was *A. sobria*. *A. sobria* was detected in 100% of intestinal samples from diseased trout. It was reported that epizootic ulcerative syndrome (EUS) caused by *A. sobria* resulted in increased mortality and morbidity to fish farms [3]. *A. sobria* was also the causative organism of fish disease in the farm of perch, *Perca fluviatilis* L, in Switzerland [4]. Chronic infections symptoms were ulceration, inflammation and skin lesions with focal hemorrhages [5] and acute septic phase induced liver and kidney damages which are the common target organs [6]. In human, *Aeromonas* spp., cause diarrhea, gastroenteritis, septicemia, wound infection, endocarditis, meningitis and pneumonia [7]. Considering the susceptibility of fish to various potential stressors, it would be advisable to conduct regular health studies on the fish. The isolated *A. sobria* was sensitive to vofloxacin, norfloxacin, rifampicin, ciprofloxacin and leucomycin [8]. Also, Hatha et al. [9] confirmed that none of the *Aeromonas* isolates were resistant to streptomycin, ciprofloxacin and nalidixic acid. Samar [10] demonstrated that clove oil could reduce total bacterial counts in water and muscle of fish toxicated by cadmium, also inhibited the growth of *Pseudomonas* and concluded that clove oil was revealed to be antioxidant in various in-vitro assays including reducing power. α,α-diphenyl-β-picrylhydrazyl (DPPH),2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and superoxide anion radical scavenging, it can be used for preventing lipid oxidation in food and pharmaceutical products reducing the formation of toxic oxidation products. The current work was planned to study the symptoms and the histopathological changes in infected African catfish (*Clarias gariepinus*) collected from various locations in Sharkia Governorate of Egypt.

Material Methods

Fish collection

One hundred and fifty (150) clinically healthy *C. gariepinus* were collected randomly with an average body weight (100-150 g) and length (20-22 cm). Fish were kept in glass aquaria (100 × 50 × 50 cm) and supplied with chlorine free water at 25 ± 1°C with electric air pumping compressors for continuous aeration(RINA, Italy). Bacterial isolation and identification:

Under aseptic conditions, biopsies were collected from different organs then cultivated on Tryptic Soya Agar (TSA) and incubated at 30°C for one day. The separated colonies were inoculated into Rimler-Shotts agar (RSA) plate for further identification. Biochemical tests were carried out according to Austin and Austin [11], and API-20E strips (BioMerieux) were used as confirmatory identification.

Pathogenicity test

Five hundred and seventy of healthy *C. gariepinus* (125 ± 5 g average body weight) were collected randomly to study the experimental infection of some bacterial strains of *A. jandaei*, *A. sobria*, *A. veronii* and *A. caviae* were isolated from naturally infected fish. The fish were acclimated for 2 weeks, and then divided into 19 equal groups.

1-Groups (1-9) were injected by 0.2 mL of 1010 *A. sobria* cells of different isolates.

2-Groups (10-12) were injected with 0.2 mL of1010 *A. caviae* cells.

3-Groups (13-15) were injected with 0.2 ml of1010 *A. jandaei* cells.

4-Groups (16-18) were injected by 0.2 mL of 1010 *A. veronii* cells.

5-Groups (19) were injected with 0.2 mL sterile saline (0.85% NaCl) as control. All experimentally injected fish were observed daily for 3-5 weeks to record any clinical signs, mortalities, abnormalities and specimens were seeded for bacterial re-isolation as determined by Miles and Misra [12].

Effect of ciprofloxacin and clove extract on mortality rate (%) of experimentally infected C gariepinus with A. sobria

To determine in vivo efficacy of ciprofloxacine and clove extract (which was detected in the previous study) against *A. sobria* infection, 120 Nile catfish (*C. gariepinus*) were divided into four equal groups each of 30. They were kept in well aerated glass aquaria measuring 100 × 50 × 50 cm to be acclimated on dechlorinated tap water for 50 days.

Group 1: Fish inoculated intra-peritoneal (IP) with 0.2 mL of 24 hours broth culture of *A. sobria* (2.5 × 108)/mL and kept without medication (positive control).

Group 2: The same of experimentally infected fish and treated with therapeutic dose of ciprofloxacin (25 mg/liter of water).

Group 3: The same of experimentally infected fish and treated with therapeutic dose of clove extract (13.25 µL).

Group 4: The same of experimentally infected fish and treated with therapeutic dose of both ciprofloxacin and clove extract.

Histopathological investigation

Tissue specimens were collected from external and internal organs as skin, gills, liver and kidney of experimentally infected fish (Table 1). Histopathological techniques were carried with reference to Carletonet research [13].

Results and Discussion

Mortality rate was among the experimentally infected *C. gariepinus* with 0. 2 mL of saline containing 1010 cells/ ml of 24 h *A. sobria* by intra-peritoneal rout (I/P) were showed in Table 1. The mortality rate was differed from 20–100% of *A. sobria* isolates. While, the mortality rate was recorded with one isolate of *A. jandaei* 90% and two isolates of *A. veronii* 20% also mortality rate in *A. caviae* range from 10-80%. Fishes were found dead without any clinical signs while the postmortem findings were congestion in liver and kidneys with hemorrhages in the intestine. *A. veronii*, *A .caviae* and *A .jandaei* were re-isolated from different organs of moribund and recently dead fish.

This variation of mortality rate was due to differences in the virulence among individual strains. The results were accordance with Sahoo et al. [14]; Wang-Kaiyu [15]; Pan-Houjun et al. [16] and Jun et al. [17] which recorded that *A. hydrophila* induced mortality ranging from 0 to 100%, while Dhanaraj et al. (2008) recorded mortality rate in experimental infected carp fish by *A. hydrophila* (45%) and reached to 100% in other fish species with the same isolates (Table 2). Mortality rate of *C. gariepinus* due to experimental infection intra-peritoneal (I/P) with 0.2 mL of 1010 cells/mL of different isolates of *Aeromonas* spp.

Ciprofloxacin, clove extract and both successfully reduced mortality rate from 83% in GP (1) to 20%, 16%, 13%, respectively by the 5th day post treatment with extract and ciprofloxacin while it completely disappeared by 7th day of treatment, this means that ciprofloxacin and clove extract was effective against MAS and the treated fish returned to the normal stats of health, our result was confirmed with those reported by authors [18-20] and also explained in detail in Table 2. The fish infected with *A. sobria* then treated by ciprofloxacin group (2) showed few peticheal hemorrhages and depression during the 1st week but some fish showed recovery at the end of experimental period this indicated that ciprofloxacin increased the survival rate of fish infected with *A. sobria* and in turn decrease the mortalities [21,22]. Fish infected with *A. sobria* then treated by clove extract group (3) showed lesions but they were milder than those in group [23]. Fish infected with *A. sobria* and treated with both Ciprofloxacin and Clove extract (4). The lesions of such group were ameliorated than those described with the infected group (1), treated with Clove extract group (3), or with Ciprofloxacin group (2) alone. These results were nearly agreed with Nascimento et al. [24], who clarified that the correlation of antibiotic and plant extract show synergistic antimicrobial activity especially with Ciprofloxacin and Erythromycin on *Pseudomonas aeruginosa*, and *Staphylococcus auras,* respectively. In Table 3, the clinical signs and the histopathological examination were recorded in the four groups.

Group 1: Catfish infected with A. sobria and non-treated

The skin shows areas of ulceration with sloughing of the epidermis, inflammatory edema and leukocyte aggregation extending to the dermis (Figure 1A). The mucous cells appeared hypertrophied with formation of intra-epidermal mucous cyst. Ballooning, hydropic and vacuolar degeneration are shown in some epidermal cells while the others show mucous metaplasia with melanomacrophages infiltration. The dermis revealed extensive aggregations of lymphocytes and edema (Figure 1B). The gills showed extensive necrosis in the covering epithelium of secondary lamellae with leukocytes replacement (Figure 2A and 2B). Congestion of the lamellar blood capillaries was noticed. Edema of the gill arch and focal hemorrhage was also seen. The suprabronchial organ also showed necrosis and leukocytes infiltration in the lining epithelium (Figure 2C). The liver revealed irregular areas of coagulative necrosis (Figure 3A). Congestion of hepatic blood vessels with few leukocytic infiltration and hemorrhage especially around the hepatoportal anastomosis (Figure 3B). The pancreatic acini were necrotic and

Table 1*. Statstical Illustrstion of infectious fishes.*

Groups	Number of fish	Items
1	30	Infected non treated
2	30	Infected and treated with ciprofloxacin (25 mg/L)
3	30	Infected and treated with clove extract (13 µ/L)
4	30	with the same previously dose Infected and treated with ciprofloxacin and clove extract

Table 2. *Mortality rate of C. gariepinus due to experimental infection intra-peritoneal (I/P) with 0.2ml of 1010 cells/ml of different isolates of A. spp.*

Items	Bacterial Isolates																		
	A.sobria									*A.cavitre*			*A. jantlaei*			*A.verotiii*			Control
Groups	1	2		4	5	6		S	9	10	11	12	13	14		16		18	19
No of fish	30	30	30						30	30	30	30	30	30			30	30	30
Organs	K	L	S	S	L	L				L		SI,		K	K	L	SL		Sterile saline
NIortality	90	100	100	—	100		100	21]	90	OD	SO	10	OD	0.0		0.0	20	20	0.0
	L: Liver; k: Kidney; S: Spleen; A: Astices.																		

Table 3. *The effect of ciprofloxacin (25mg /L) & clove extract (13. 25 µ/L) on mortality rate (%) of experimentally infected Clarias gariepinus with A. sobria.*

Fish grouping	Total number	Number of dead fish	Mortality 0/0
G(1) Infected non treated	30	25	83
G(2)Infected and treated with ciprofloxacin	30	6	20
G(3)Infected and treated with clove extract	30	5	16

infiltrated with lymphocytes. The kidney showed coagulative necrosis in the tubules, congestion of the blood vessels and depletion of the hemopoietic elements (Figure 4). The glomeruli were contracted with dilated Bowman's capsule, similar results were obtained by authors [25-28].

Group 2: Catfish infected with A. sobria and treated with ciprofloxacin

The skin shows intact epidermis with slight increase in mucous cells and few leukocytes infiltration extending to the dermis (Figure 5). The underlying muscles were rarely suffering from necrosis. The gill showed congested blood capillaries and focally desquamated epithelium at the tips of the primary lamellae (Figure 6). The liver showed hydropic degeneration and vacuolations in the hepatocytes (Figure 7). Hyperemia of the hepatic blood vessels and hemorrhage were rarely detected. The kidney revealed focal vacuolations in the tubular epithelium with activation in the hemopoietic cells (Figure 8). Hypercellularity in the glomerular tufts was noticed. Few PNLs were seen in the interstitial tissue. Cellular and hyaline casts were observed in the lumina of some renal tubules, this result corresponded with the authors which indicated the ciprofloxacin increases the survival rate of fish challenged with *A. sobria* and in turn decrease the mortalities [21,22].

Group 3: Catfish infected with A. sobria and treated with clove extract

The lesions in this group were similar to those described in group (1) but they were milder. The skin showed extensive necrosis in the epidermis with intense lymphocytic infiltration. The remaining epidermal cells undergo spongiosis and ballooning degeneration with increased and hypertrophied mucous cells (Figure 9A). The epidermis, dermis and skeletal muscles were invaded by numerous granulocytes and few lymphocytes (Figure 9B). The skin was edematous and the muscle bundles were degenerated and focally necrotic. The gill showed hypertrophy and hyperplasia of the mucous cells with fusion of the gill filaments was focally noticed with leukocytes infiltration (Figure 10A). Congestion of branchial blood vessels and extravasation of erythrocytes (Figure 10B). The lining epithelium of the secondary lamellae undergoes focal proliferation, frequent desquamation, focal destruction and lymphocytes infiltration (Figure 10C). The liver revealed congestion of hepatic sinusoids beside diffuse hydropic changes in the hepatocytes and few leukocytes infiltration (Figure11A). Focal macrovesicular steatosis and coagulative necrosis were also noticed in the hepatocytes (Figure 11B). The pancreatic acini were focally necrotic with depletion of zymogene-granular acini. The kidney showed focal areas of coagulative necrosis in the tubules with activation of the hemopoietic elements (Figure 12). The glomeruli were destructed with multifocal areas of hemorrhage [23].

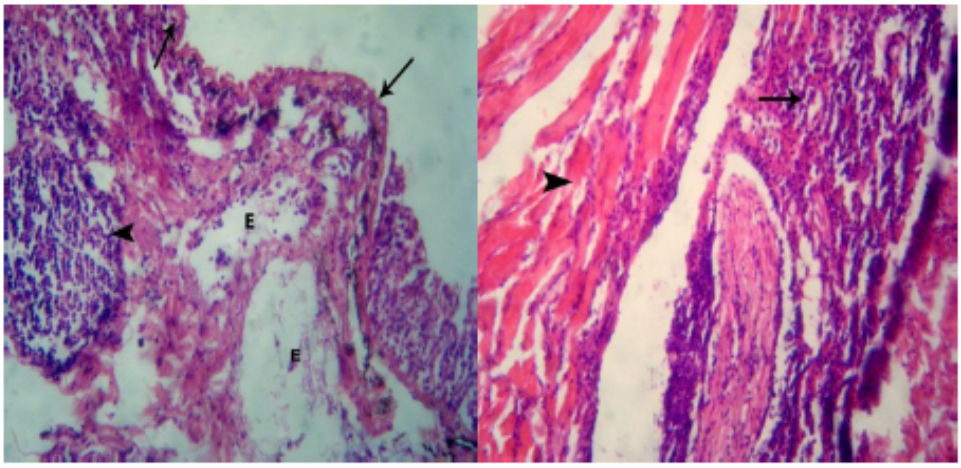
Group 4: Catfish infected with A.sobria and treated with the synergistic effect of ciprofloxacin and clove extract

The skin showed intact epidermis with no evidence of erosion and ulceration while the dermis was infiltrated by few lymphocytes and the muscle fibers were widely separated by edema (Figure 13). The gills revealed mild proliferation of the covering epithelium particularly at the base of the secondary lamellae and few lymphocytes infiltration (Figure 14). Congestion and hemorrhage are rarely visualized. The liver showed intact parenchyma with slight congestion, few interstitial and portal lymphocytes infiltration, focal hydropic degeneration and vacuolations of some hepatocytes (Figure 15). The kidney was normal except few focal vacuolations in the tubular epithelium (Figure 16). The lesions of fish infected with *A.sobria* and treated by both ciprofloxacin and clove extract were ameliorated than those infected with *A.sobria* and treated by either ciprofloxacin or clove as described by Nascimento et al. [24].

Deivasigamani [29] concluded that infected catfish mortality is due to typical histopathological lesions such as necrosis of hepatocytes, sheathed arteries in the spleen and renal tubules and glomeruli.

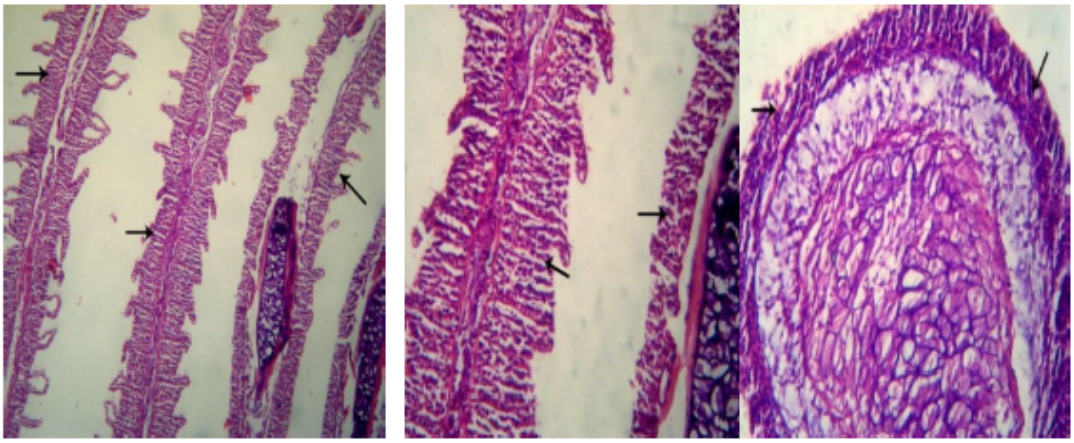
Conclusion

This result shows both ciprofloxacin and clove extract was the best choice as an alternative bacterial therapeutic agent in infected *Clariasgariepinus* with *A.sobria*. Since *A.sobria* may threaten public health, transmission of the reduced susceptibility maybe has negative consequences for humans.



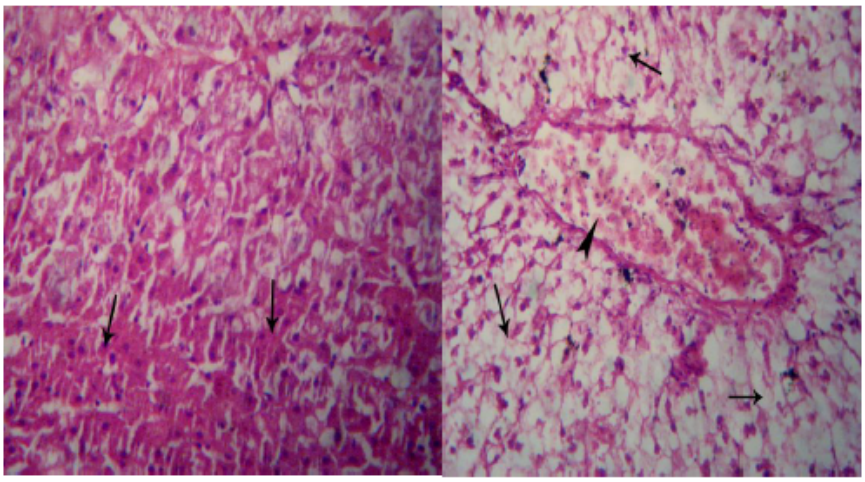
A *B*

Figure 1. *(A) Skin section of C. gariepinus from group 1 showing area of ulceration (arrow) and aggregation of leukocytes (arrowhead) and (B) showing aggregation of lymphocytes (arrow) and Zenker's necrosis in skeletalmuscles (arrowhead) in the dermis. (H&E stain × 400).*



A *B* *C*

Figure 2. *(A&B) Gill section of C.gariepinusfrom group 1 showing e×tensive necrosis in the covering epithelium of the secondary lamellae with leukocytes aggregation (arrows) (H&E,× 100) and (C) showing necrosis and leukocytes infiltration in the epithelium of suprabronchial organ (arrows) (H&E, × 400).*



A *B*

Figure 3. *(A) Liver section of C.gariepinus from group 1 showing coagulative necrosis (arrows) and (B) showing congestion (arrowhead) and diffuse vacuolations of hepatocytes (arrows) (H&E,× 400).*

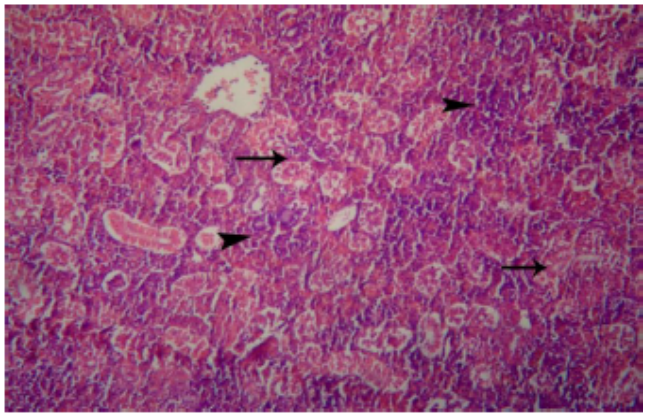


Figure 4. *Kidney section of C.gariepinus from group 1 showing severe coagulative necrosis in the renal tubules (arrows) and depletion of hemopoietic elements (arrowheads) (H&E stain × 100).*

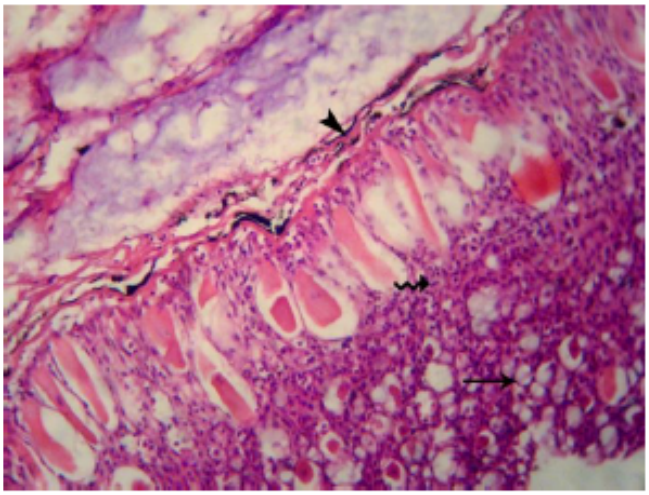


Figure 5. *Skin section of Clarias gariepinus from group 2 showing intact epidermis and slight increase in mucous cells (arrow), ballooning degeneration in the epidermal cells and few leukocytes infiltration. (H&E stain × 400).*

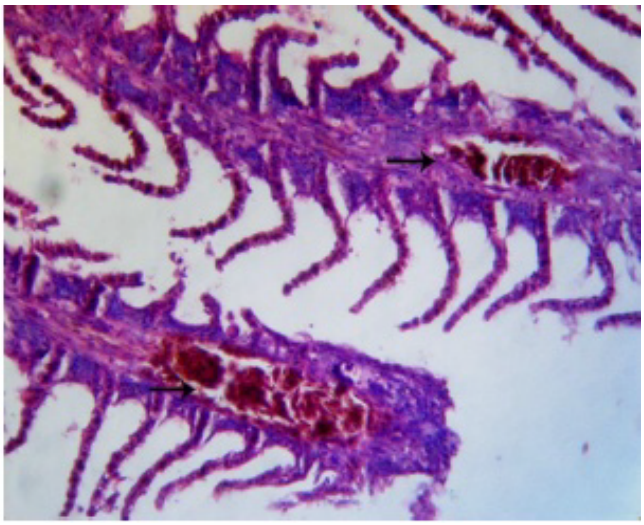


Figure 6. *Gill section of Clariasgariepinusfrom group 2 showing congested blood capillaries (arrow) and the tips of the primary lamellae were focally desquamated from the lining epithelium. (H&E stain × 100).*

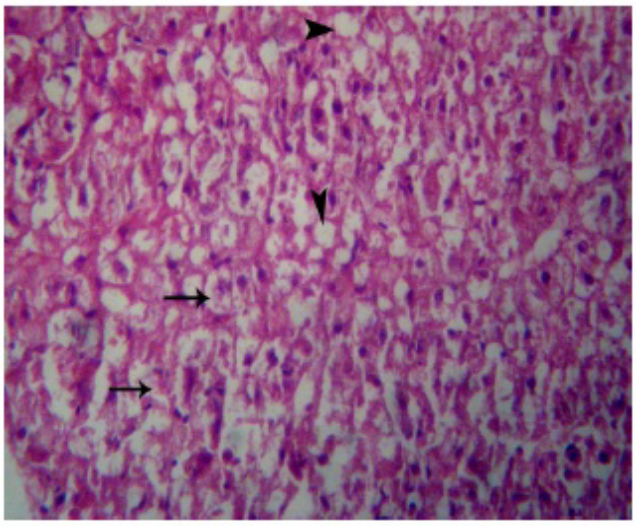


Figure 7. *Liver section of Clariasgariepinusfrom group 2 showing hydropic degeneration and vacuolations in the hepatocytes (arrow). (H&E stain × 400).*

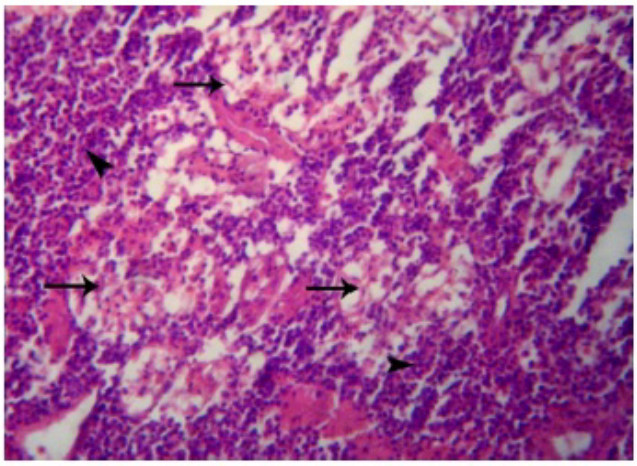
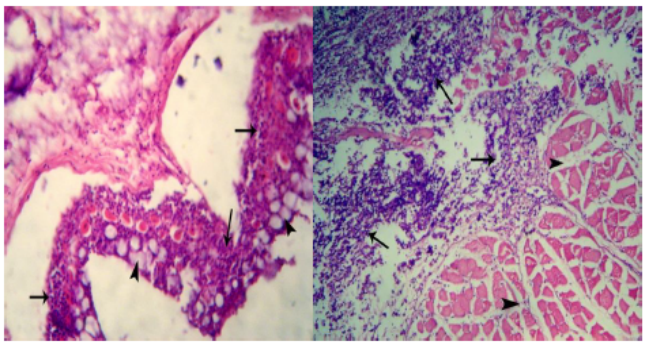
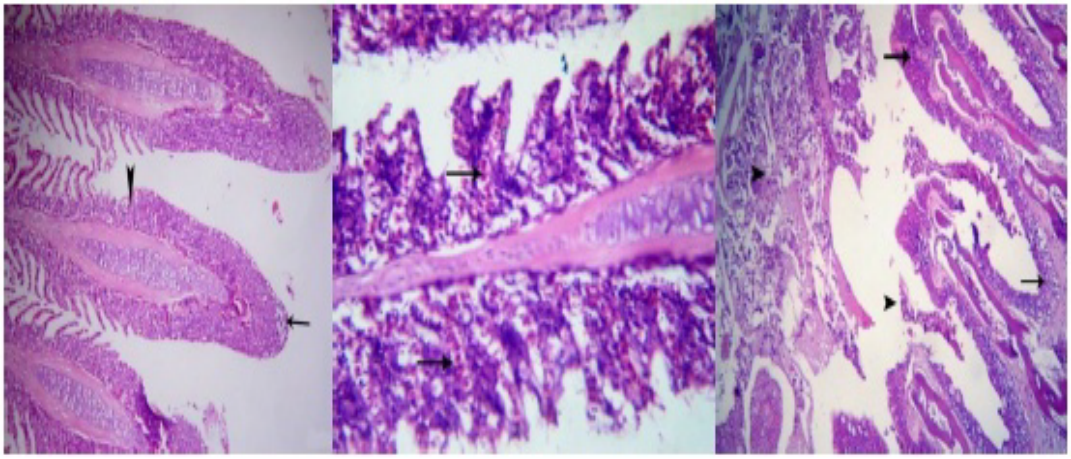


Figure 8. *Kidney section of Clariasgariepinusfrom group 2 showing focal vacuolations in the tubular epithelium (arrow) and activation in the hemopoietic cells (arrowheads). (H&E stain × 400).*



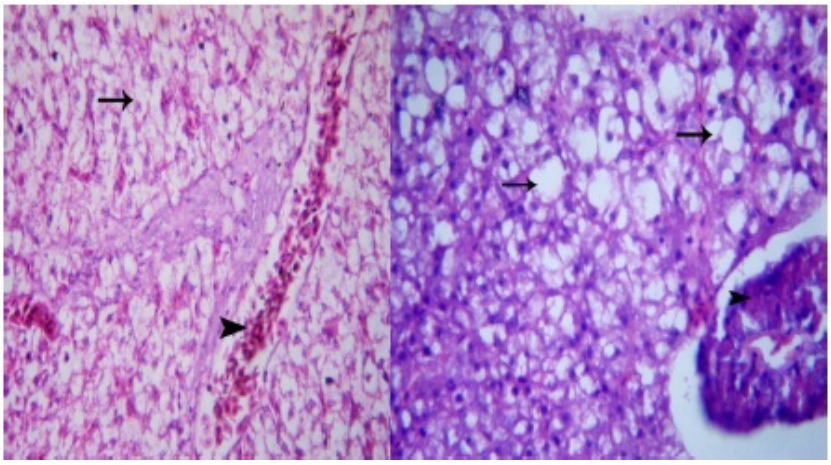
A *B*

Figure 9. *(A) Skin section of Clariasgariepinusfrom group 3 showing e×tensive necrosis in the epidermis with intense lymphocytic infiltration (arrow) and increased the mucous cells (arrowheads). (H&E stain × 400) and (B) intense aggregation of lymphocytes in the dermis (arrows) and among the skeletal muscles. (H&E stain × 200).*



A *B* *C*

Figure 10. *(A) Gill sections of Clarias gariepinus from group 3 showing proliferation of the epithelial covering of the secondary lamellae and leukocytic infiltration (arrow). (H&E stain × 200), (B) congestion and hemorrhage (arrows). (H&E stain × 400) and (C) Gill rakers with severe inflammation, mucinous degeneration (arrows) and e×tensive desquamation in the lining epithelium and leukocytic infiltration (arrowheads). (H&E stain × 100).*



A ***B***

Figure 11. *(A) Liver section of Clarias gariepinus from group (3) showing severe congestion (arrowhead) and vacuolations in the hepatocytes (arrows) and (B) showing macrovesicular steatosis (arrows). (H&E stain × 400).*

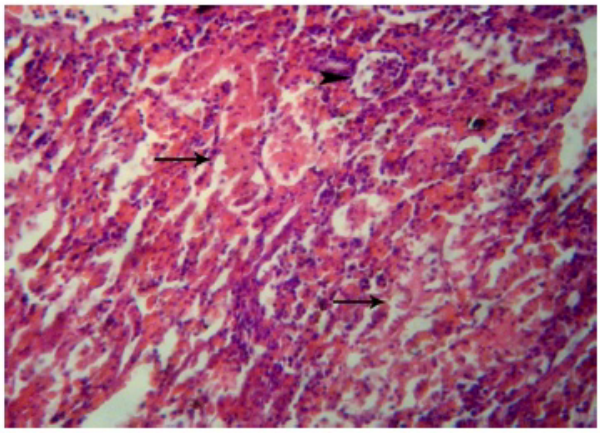


Figure 12. *Kidney section of Clariasgariepinusfrom group 3 showing focal coagulative necrosis in the renal tubules (arrows) and activation of the hemopoietic elements. (H&E stain × 400).*

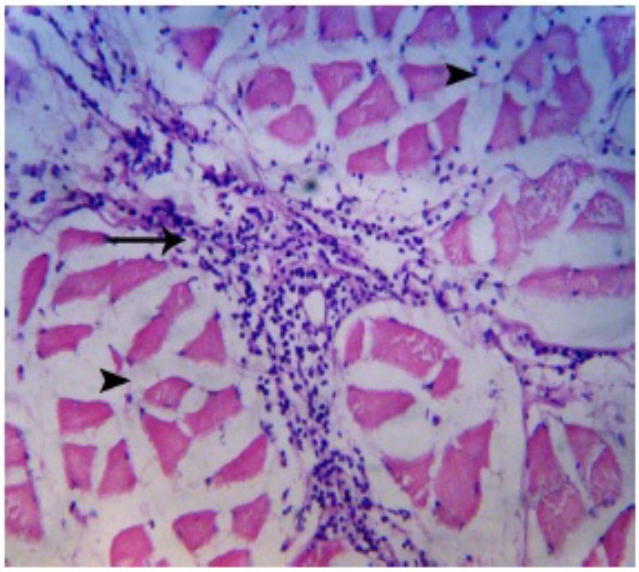


Figure 13. *Skin section of Clarias gariepinus from group 4 showing few lymphocytes infiltration among the muscle in the dermis (arrow). (H&E stain × 200).*

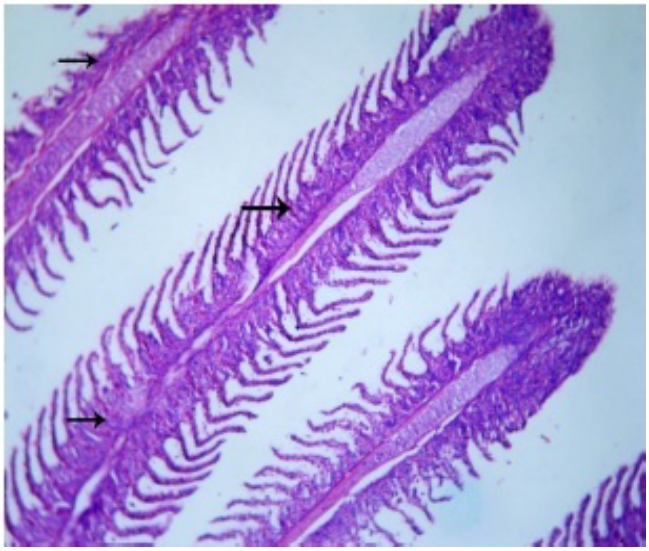


Figure 14. *Gill section of Clarias gariepinus from group 4 showing mild proliferation of the covering epithelium at the base of the secondary lamellae (arrows). (H&E stain × 100).*

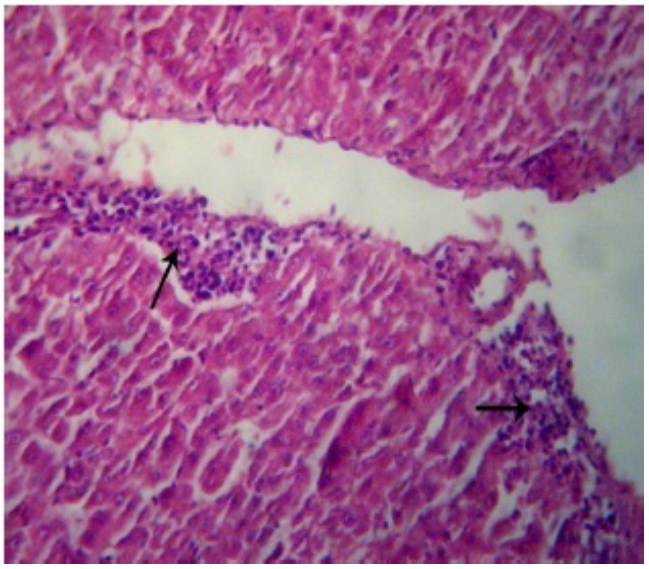


Figure 15. *Liver section of Clariasgariepinusfrom group 4 showing few lymphocytes in the portal area (arrows). (H&E stain × 400).*

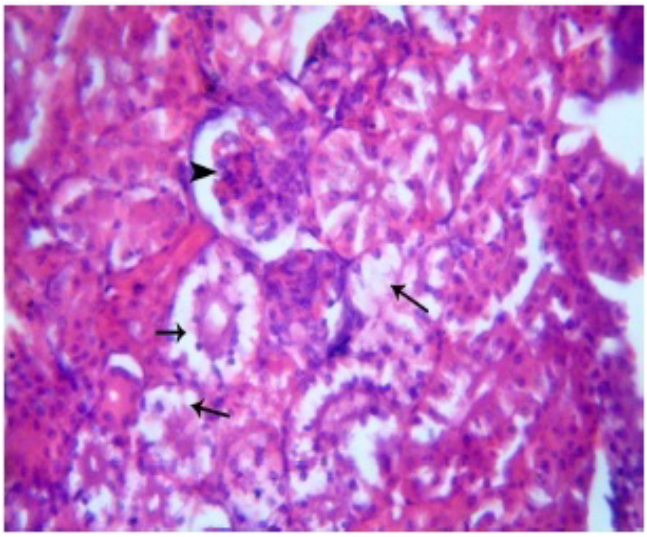


Figure 16. *Kidney section of Clariasgariepinusfrom group (4) showing mild vacuolations in the tubular epithelium (arrows) and congested glomerulus (arrowheads). (H&E stain × 400).*

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