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Investigation the effect of *camallanus* spps infestation on hematological, immuno-biochemical and histological changes in catfish (*Clarias gariepenus*) with trials of treatment

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Abstract
The present study was carried out to investigate the effect of *camallanus spps* infestation on hematological, immuno-biochemical parameter and on histological pictures in catfish (*Clarias gariepenus*) with trials of treatment by using levamisole and fenbendazole. A total 100, catfish were divided into 4 equal groups (25 fish in each). The 1st group clinically healthy fish served as (control group). The 2nd group was naturally infested fish with *camallanus* and non-treated. The 3rd and 4th groups were naturally infested and treated with levamisole and fenbendazole respectively. Three blood samples were collected from each fish in all group post treatment (PT) at the end of 1st and 4th weeks for hematological and immuno-biochemical studies. After recording clinical signs and post-mortem (PM) findings. Specimens were collected from all groups at the end of 4th weeks PT from liver, kidney, intestine, spleen and gills for histopathological examination. Parasitological examination of catfish revealed presence of *camallanus* species that is identified as red color; smooth, cylindrical and short round worms and their location toward the posterior of intestinal tract than other worm like parasites.
The results of infested fish with *camallanus* spps revealed a significant increase in TLC, eosinophils, IgM, IgD, C RP, AST, ALT, uric acid and creatinine. Meanwhile a significant decrease in heterophiles, lymphocytes, monocytes, phagocytic % and index, total proteins, albumin and globulin when compared with control group throughout the experimental period. The macroscopical & microscopical findings were recorded. Infested catfish treated with levamisole and fenbendazole in groups (Gp3& 4) showed reduction in clinical signs and an improvement in hematological and immuno-biochemical parameters with an improvement in the pathological changes at the end of 4th week PT. It could be concluded that the levamisole is highly effective than fenbendazole against *camallanus* infestation in catfish and ameliorating its adverse effect on hematological, immunological, biochemical parameters and on pathological changes.

Keywords: *Catfish (Clarias gariepenus), Nematodes, Camallanus spps, Levamisole, Fenbendazole, Hematological, Immunological, Biochemical Parameters and Histopathological Changes.*

Introduction

Fish often comprises a nutritionally important part of many people's diets in developing countries. It is avital source of protein and micronutrients, which improves the quality of protein in largely vegetable and starch based diets by providing essential amino acids **(Osman *et al*., 2018).** The parasitic infestation all over the world and under our Egyptian condition are one of important limiting factors of fish productivity and reproductively **(Anthony *et al*., 2014).** Parasitic infestations especially gastrointestinal nematode of fish resulted in decrease in trace element and vitamins, destruction of gastrointestinal mucosa and massive mortality in high-density aquaculture sys-

tems also possess serious health threat and retarder fish productivity, **(Adawy *et al*., 2012).** Fish macroscopic parasites are among the major pathogens, caused fish diseases and spoil the appearance of fish resulting in consumer rejection (**Abdel-Gaber *et al.* 2015).** Fish nematodes regarded as one of the most important and harmful worm parasites deprive their host food and feed on host tissues, sera and blood causing emaciation and anemia in fish **(Nnabuchi *et al*., 2015).** Nematodes are known as round worms like slender, unsegmented worms, particularly in aquatic environment that infest fish directly without need intermediate host or indirect life cycles **(levsen 2001).** *Camallanus* and *capillaria* are the most common nematodes affecting fishes found in wild fresh and saltwater fishes throughout the world (**Mai 2018).** Genus *camallanus* is smooth, cylindrical and short roundworm than the *capillaria*, have indirect life cycles live-bearing nematode as incubate eggs/larvae within worm bodies females "ovoviviparous" **(Yanong 2011).** The hatched larvae excreted into water with fish's feces which ingested by aquatic second vectors such as (copepod, tubifex worm or insect) and developed to 3rdstage larvae, which entering fish as final definitive host **(Okoye *et al*., 2016).** Larvae migrate out to fish gut and develop into reproductive adults and the life cycle is complete inducing sever internal pathological lesions of fish where injuries the lumen of intestinal wall (**Ruhela *et al* 2012).** The most common and effective drugs for *camallanus* spps are levamisole and fenbendazole, both drugs are specific for killing their roundworm target and have a wide safety margin as fenbendazole powder 250 mg is added to ration while levamisole 2 ml can be added to either ration or water (**Diana Walstad 2017).**

This study aimed to investigate the adverse effect of *camallanus* infestation on hematological, immuno-biochemical parameters and on histological picture in catfish (*clarias gariepenus*) with trials of treatment with levamisole and fenbendazole.

Materials and Methods

1-Materials

A. Fish samples

A total, 100 catfish (*Clarias gariepenus*) of different sexes and weighting (150 g ± 50-gram body weight) were collected from branch of Nile River in Sharkia governorate, and then sported under the hygienic measurements. Fish were maintained in well-aerated glass aquaria (about 52 liters capacity) (40x 40x80 cm each) filled with de-chlorinated tap water of about PH (7.2-8.5) and 25 ^{0}C temperature **(Roberts, 2012).** Cleaning of de-chlorinated water was carried every three days **(Brengun, 2009)**. Fish fed on a base diet along the experimental period. Fish were acclimatized to laboratory conditions for a week under strict hygienic measures, before the beginning of the experiment.

Parasitological examination of fish samples:

a- Fecal Examination: Feces was collected from the fish by first gently wiping the moisture from the bellies of the fish with a paper towel. Then using a moderate squeezing motion with the thumb and forefinger pressure was applied to the abdomen, starting in line at the front of the pelvic fins and finishing at the anus. Feces collected in a petri dish according to **Scott and Pamela (2015) and Hoffman, G.L. (2019)**.

b- Necropsies (Internal) examination: was carried to each fish for helminthes infestations. The examination involved the skin, fins, gills, buccal cavity and alimentary tract of fish, which was left for a few minutes into a petri dish containing saline solution then opened, scraped and examined under dissecting microscope **(Lucky1977)**.

Fixation, Staining and Identification of Parasites: Nematodes were fixed by using ethyl alcohol (70%) directly after collection. Nematodes gave the best results without staining but cleaning in Lacto phenol, gave good results as a clearing material with nematodes and polyol were used as mounting materials **(Lucky 1977).** The collected nematode are identified **(Schludermann *et al*., 2003 and Nnabuchi *et al*., 2015).**

B. Water sample

Parasitological examination of water sample: was carried for second vector as (cyclops, copepod or other crustacean) which contain

3rdstage larvae of *camallanus* (**Zhokhov and Mironovsky 2007).**

Chemical examination of water sample: pH value were measured by **JENWAY (pH meter). Model**: 3510. Ammonia (NH4/NH3) by **Spectrophotometer**. **Model: 690** according to **Marie *et al*., (1993**). Nitrite according to **Zhang *et al*., (2016)**. Total Dissolved Solids salts (T.D.S) calculated according to this equation**:** W2-W1 X 10, as w1= weight of empty flask and W2= weight of flask after heating with 100 ml water until boiling then evaporated all water and remaining salts only**.**

Drugs:

Levamisole HCL: The hydrochloride salt of levamisole is water-soluble form, broad-spectrum anthelmintic drug. Once infested fish stopped eating, the water is only real treatment option and fish readily absorb levamisole from their skin and gills into blood stream (**Chandy *et al*., 2016).** The recommended dose for treating by levamisole 2 ml / liter water twice in a week and then repeating treatment again after 2 weeks later **(Diana Walstad 2017).** Levamisole: Produced by EL Nasr Pharmaceutical Chemicals Co. Egypt. Abu- Zaabal.

Fenbendazole: (Fish Bendazole) 2.5%: is insoluble form, broad-spectrum anthelmintic drug used in treatment, kill and control of gastrointestinal nematodes **(Dayan 2003).** The recommended dose for treating by fenbendazole powder 250 mg / kg ration. Treatment occurred by thoroughly mixing ration with fenbendazole 250 mg / kg ration at least once a day for a week and then repeating treatment again after 2-3 weeks later **(Diana Walstad 2017).** Fenbendazole: (OPIZOLE) 2.5%: Suspension. Produced by EL- Obour Modern Pharmaceutical Industries Co. Egypt. EL- obour City 1st Industrial Area.

2-Methods

Experimental design

One hundred live catfish were examined clinically and parasitological, we found 25 apparently healthy served as the first group (Gp1) control and 75 are infested groups by *camallanus,* That divided into 3 equal groups (25 in each). Second group (Gp2) infested non-treated catfish. Third group (Gp3) infested catfish and treated with levamisole 2 ml / liter water twice a week then repeating the treatment after 2 weeks later **(Diana Walstad 2017)**. Fourth group (Gp4) infested catfish were treated with fenbendazole by well incorporated with 250 mg / kg ration once daily for a week and then repeating the treatment after 2 weeks later **(Noga 2010)**.

Blood samples: Three blood samples were collected from each fish in the each groups via caudal vein under aseptic condition directly at the end of 1st and 4th weeks PT for hematological and immuno-biochemical examination.

First sample was taken on a tube contained EDTA for hematological estimation **(Jain, 1993).**

Second sample was collected on heparinized tube for phagocytosis assay.

A- Separation of Peripheral Blood Mononuclear Cells (PBMC) using ficoll-plaque density gradient was carried out **(Boyum 1986 and Godeeris *et al.*, 1986).**

B- Phagocytic assay **(Wilikinson 1976)**

C- Measurement of phagocytic activity of Peripheral Blood Monocyte **(**PBM**)** using *Candida albicans* was performed **(Anthony *et al*., 1985 and Chu and Dietert 1989).**

D- Evaluation of phagocytic activity **(Wilikinson 1976).**

Third blood sample was taken without anticoagulant in a clean dry centrifuge tube to obtain clear serum and using spectrophotometer for estimation: Total protein **(Doumas *et al.*, 1981).** Albumin **(Drupt, 1974)** and Globulin (calculated as difference between T. proteins and albumin). AST- ALT **(Reitman and Frankel, 1957).** ALP (**Tietz, 1996).** Uric acid **(Sanders *et al*., 1980)**. Creatinine **(Henry, 1974).** C- reactive protein (CRP) (**Nathan and Scheld 2002)** and immunoglobulins titer: IgM & IgD by development of the sandwich ELISA **(Erhard *et al*., 1992).**

Histopathological Studies: Specimens were collected from liver, kidneys, spleen, gills and intestine from all groups at the end of 4th weeks PT after recording post-mortem (PM) examinations. They were fixed in 10 % neutral buffered formalin solution then dehydrated, cleared and embedded in paraffin wax then specimens were sections to 4-5 micron thickness were prepared and stained with hematoxylin and eosin (H &E) and examined microscopically **(Survarna *et al.*, 2013).**

Statistical Analysis: The obtained data was computerized using one-way (ANOVA) test. Duncan's Multiple Range, calculation of standard error and variance according to **SPSS 14 Version (2006).**

Results

Clinical signs (examination) of catfish infested with *camallanus* spps showed off food, emaciation, opacity of eyes, deformed body shape, grayish ulcers patches on dark skin, ulceration of gill cover, fraying of fins, ascites, distension of the abdomen, reduced swimming performance **(Photo, 1) and (Fig, 1).** Fish slow in motion, floating on their sides on water surface. Death some fish were showed in 2nd-group.

Photo. (1): Main clinical signs grayish ulcers on catfish skin infested with *camallanus.*

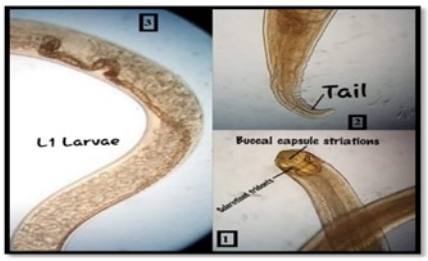

Photo. (2): (1) Anterior end of *camallanus sp.* showing buccal capsule striations and sclerotized tridents.
(2) Posterior end of *camallanus sp.* showing male tail.
(3) Median body thin of *camallanus sp.*

Parasitological examination of fish revealed presence of *camallanus* species that is identified as red color, their location toward the posterior of intestinal tract than other worm like parasites. Characteristic features, of genus *camallanus* is smooth, cylindrical and short round worms (**Photo, 2).** It,s easily recognized as a small reddish thread-like worm protruding from the anus of the fish. Microscopic examination appears presence of buccal capsule of mouth structure divided into two lateral valves, giving the mouth a slit like appearance, presence of both eggs and larvae within worm body (ovoviviparous), **(Photo, 2).**

Parasitological examination of water sample for aquatic second vectors, which contain 3rd-stage larvae of *camallanus.*

Chemical examination of water quality parameter showed pH value = 8.45. Ammonia (NH4/NH3) = 0.20 ppm. Nitrite = 0.02 ppm. Total Dissolved Solids salts (T.D.S) = 120 ppm.

Table (1). Some chemical water analysis.

Examination	Result	Reference value according to WHO (1993)
pH	8.45	6.50 -8.50
Ammonia(NH_4/NH_3)	0.20 ppm	0.50 ppm>
Nitrate	0.02 ppm	0.10 ppm>
T.D.S	120 ppm	500 ppm

Hematological results:

Leukogram: TLC & Eosinophil showed a significant increase in groups (Gp2, 3 &4) at 1st and 2nd collection, but non- significant change in (Gp3) at 2nd collection as compared to control group. **Lymphocytes and Monocytes:** showed a significant decrease in groups (Gp2, 3 &4) at 1st collection while insignificant change in groups (Gp3& 4) at 2nd collection as compared to control group. **Heterophiles:** showed a significant decrease in groups (Gp2, 3 &4) at 1st and 2nd collection, but insignificant change in (Gp3) at 2nd collection as compared to control group. **Basophiles** showed a significant decrease in (Gp2) at 1st collection and non-significant change at 2nd collection also non-significant change in groups (Gp3 &4) at 1st and 2nd collection as compared to control group **(Table, 2).**

Cellular immunity: Phagocytosis were showed a significant decrease in both phagocytic % and index in groups (Gp2, 3 &4) at 1st and 2nd collection, while insignificant change in (Gp3) at the end 2nd collection as compared to control group **(Table, 3)**.

Biochemical analysis: Total Proteins (TP), **albumin and globulin** showed a significant decrease in-group (Gp2) at 1st and 2nd collection and groups (Gp3 & 4) at 1st collection only with non-significant change at 2nd collection as compared to control group. **A/G Ratio:** showed a significant decrease in (Gp2) at 1st and 2nd collection but insignificant change in groups (Gp3& 4) at 1st and 2nd collection as compared to control group. **CRP:** Showed a significant increase in all groups at 1st and 2nd collection as compared to control group **(Table, 4).**

Humoral immune response: Immunoglobulin M **(IgM)** and Immunoglobulin D **(IgD)** showed a significant increase in groups (Gp2, 3 &4) at 1st collection. A significant increase in (Gp2) with insignificant change in (Gp3 & 4) at 2nd collection as compared to control group **(Table, 4).**

Liver enzymes activities: Alanine aminotransferase **(ALT)** and Aspartate aminotransferase **(AST)** showed a significant increase in all groups at 1st collection. Significant increase in (Gp2), while insignificant change in (Gp3&4) at2nd collection as compared to control group **(Table, 5).**

Kidneys function tests: Uric acid: showed a significant increase in groups (Gp2, 3 &4) at 1st and 2nd collection. Except insignificant change in (Gp3) at 2nd collectionas compared to control group. **Creatinine:** showed a significant increase in groups (Gp2, 3 &4) at 1st collection. Significant increase in (Gp2), while non-significant change in (Gp3&4) at 2nd collection as compared to control group **(Table, 5).**

Pathological studies:

The gross examination of fish in (Gp2) revealed discoloration of skin accompanied with different ulcers on dark skin and paler gills **(Fig, 1- A). PM examination** showed pericarditis, perihepatitis, stomatitis, enteritis, splenomegaly and nephritis. In addition to congestion of all internal organs, enlarged gall bladder and pale yellowish. Liver enlarged and somewhat congestion with petechial hemorrhages. Kidneys were swollen, dark red and friable. Spleen was enlarged. Stomach was distended. The intestine were congested and had a watery yellowish mucous. Paler gills **(Fig, 1-D).** While the treated (Gp3 & 4) revealed mild macroscopical changes.

Microscopically:

Liver showed dilated portal tract with short fibrous septa extends to the hepatic parenchyma and congested portal vein **(Fig, 2-C).** Dilated thin walled and congested central vein surrounded by hepatocytes with pale granular eosinophilic cytoplasm and bland rounded nuclei. Dilated, portal area with inflammatory cells **(Fig, 2-B)** in (Gp2). While in (Gp3) liver showed thin walled dilated and congested central veins surrounded by intact hepatocytes with pale granular eosinophilic cytoplasm. Dilated and congested central vein with thin fibrous band extend to the parenchyma and infiltrated by mild lymphocytic extended infiltration **(Fig, 2-C)**, but in (Gp4), expanded by fibrosis and surrounded by intact hepatocytes. Mildly dilated central vein surrounded by few degenerated hepatocytes **(Fig, 2-D)**. **Kidneys of the second group** revealed thickened renal blood vessels with narrow lumen surrounded by mononuclear inflammatory cells and degenerated renal tubules **(Fig, 2-F).** Heavy stromal mononuclear inflammatory cellular infiltrates and degenerated epithelial lining renal tubules

(Fig, 3-A), but in (Gp3) revealed moderate stromal lymphocytic infiltrate, moderate hemosiderin deposition and vacuolar degeneration of the renal tubules. While in (Gp4) revealed markedly degenerated epithelial lining renal tubules **(Fig, 3-B) & (Fig, 3-C). Spleen** showed splenomegaly with congestion in white and red pulps of 2nd group **(Fig, 2-E)**. **Intestine** of **(Gp2)** revealed larval stage of parasite in the wall of intestine **(Fig, 3-D), &** with hyperplasia of the goblet cells and lymphocytic infiltration in lamina propria were recorded in all groups **(Fig, 3-E).** Intestine of (Gp4) infested catfish with camallanous and treated with fenbendazole showing hyperplasia of the goblet cells and lymphocytic infiltration in lamina propria **(Fig, 3-F). Gills of (Gp2)** infested non treated catfish with camallanous showed degeneration of primary lamellae of gills with absence of secondary lamellae of the gills of infested groug **(Fig, 1-E)**. Levamisole and fenbendazole treatment improved the pathological lesions in spleen, intestine and gills.

Table (2). Effect of levamisole and fenbendazole on leukogram of naturally infested catfish with *camallanus* (mean values ± S.E) (n=5).

Parameters / Sampling Groups	TLC (10 3×mm 3)		Lymphocytes %		Hetrophils %		Monocytes %		Basophils %		Eosinophils %	
	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT
(Gp1) Control	16.72 ± 0.037 d	18.84 ± 0.092 c	61.36 ± 0.11 a	61.8 ± 0.35 a	32.32 ± 0.037 a	31.26 ± 0.36 a	4.92 ± 0.037 a	5.64 ± 0.18 a	0.43 ± 0.19 b	0.30 ± 0.18 a	1 ± 0.0 d	1± 0.18 c
(Gp2) Camallanus Infested	25.82 ± 0.092 a	31.1 ± 0.33 a	58.80 ± 0.06 d	59.68 ± 0.38 b	31.6 ± 0.19 b	27.65 ± 0.27 c	3.38 ± 0.037 c	3.16 ± 0.17 b	0.00 ± 0.0 c	0.96 ± 0.28 a	6.22 ± 0.097 a	8.55 ± 0.17 a
(Gp3) Levamisole Treated	19.46 ± 0.163 c	18.96 ± 0.32 c	60.53 ± 0.17 b	62.04 ± 0.40 a	30.82 ± 0.066 c	30.86 ± 0.34 a	3.9 ± 0.032 b	4.9 ± 0.24 a	0.45 ± 0.17 b	0.80 ± 0.37 a	4.30 ± 0.032 c	1.4 ± 0.37 c
(Gp4) Fenbendazol Treated	22.04 ± 0.262 b	20.48 ± 0.17 b	59.64 ± 0.68 c	62.34 ± 0.26 a	31.1 ± 0.105 b	29.40 ± 0.18 b	3.46 ± 0.024 c	5.30 ± 0.18 a	0.50 ± 0.00 b	0.90 ± 0.10 a	5.30 ± 0.089 b	2.06± 0.17 b

Different letters in the same column means significant difference at ($p \leq 0.05$)
Gp = Groups W= week PT= post treatment.

Table (3). Effect of levamisole and fenbendazole on phagocytic activity (% & index) on naturally infested catfish with camallanus (mean values ± S.E) (n=5).

Parameter / Sampling Groups	Phagocytic %		Phagocytic index	
	1st W PT	4th W PT	1st W PT	4th W PT
(Gp1) Control	73±0.71 a	73.8 ± 0.58 a	4.3 ±0.032 a	4.45 ± 0.022 a
(Gp2) Camallanus Infestated	51 ±0.45 d	47.6 ± 0.75 c	2.1 ±0.045 d	1.8 ± 0.037 c
(Gp3) Levamisole Treated	66.8 ±0.56 b	75 ± 1.00 a	3.32 ± 0.051 b	4.47 ± 0.043 a
(Gp4) Fenbendazol Treated	60 ±0.37 c	70.6 ± 0.75 b	2.99 ±0.033 c	4.19 ± 0.064 b

Different letters in the same column means significant difference at ($p \leq 0.05$) PT= post treated G = Groups W= week

Table (4). Effect of levamisole and fenbendazole on proteinogram and humoral immune response of naturally infested catfish with *camallanus* (mean values ± S.E)

Parameters / Sampling Groups	T. Protein g/dl		Albumin g/dl		Globulin g/dl		Albumin/ Globulin Ratio		C. Reactive Protein ng/ml		Immunoglobulin (IgM) ng/ml		Immunoglobulin (IgD) ng/ml	
	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT	1st W PT	4th W PT
(Gp1) Control	3.2 ± 0.008 a	3.28 ± 0.015 a	1.18 ± 0.008 a	1.21 ± 0.009 a	2.026 ± 0.008 a	2.07 ± 0.006 a	0.6 ± 0.00 a	0.60 ± 0.00 a	31 ± 0.58 d	33 ± 0.58 c	5.27 ± 0.43 d	9 ± 0.58 b	26 ± 1.15 d	33 ± 1.15 c
(Gp2) Camallanus Infested	2.62 ± 0.012 d	2.50 ± 0.003 d	0.89 ± 0.021 c	0.84 ± 0.012 b	1.73 ± 0.015 d	1.66 ± 0.008 c	0.52 ± 0.015 b	0.50 ± 0.003 b	119 ± 0.58 a	111 ± 5.69 a	66.0 ± 0.58 a	72 ± 1.73 a	237 ± 1.73 a	252 ± 1.76 a
(Gp3) Levamisol Treated	2.90 ± 0.015 b	3.20 ± 0.010 a	1.05 ± 0.029 b	1.18 ± 0.020 a	1.85 ± 0.029 c	2.02 ± 0.074 a	0.57 ± 0.02 a	0.58 ± 0.033 a	52 ± 1.15 c	35 ± 1.15 c	21.0 ± 0.58 c	11 ± 1.15 b	79 ± 0.58 c	35 ± 0.58 bc
(Gp4) Fenbendazol Treated	2.82 ± 0.014 c	3.19 ± 0.006 a	1.03 ± 0.015 b	1.22 ± 0.025 a	1.79 ± 0.017 b	1.97 ± 0.017 a	0.58 ± 0.023 a	0.62 ± 0.00 a	69 ± 0.57 b	48 ± 1.15 b	31.0 ± 0.57 b	12 ± 0.58 b	109 ± 0.58 b	38 ± 1.15 b

Different letters in the same column means significant difference at ($p \leq 0.05$) W= week PT= post treated.

Table (5). Effect of levamisole and fenbendazole on liver enzymes activities and kidneys function test of naturally infested catfish with *camallanus* (mean values ± S.E) (n=5).

Parameters / Sampling Groups	ALT U/L		AST U/L		Uric acid mg/dl		Creatinine mg/dl	
	1st W PT	4thW PT	1st W PT	4thW PT	1st W PT	4th W PT	1st W PT	4thW PT
(Gp1) Control	36± 0.29 d	38.67± 0.88 b	106.07± 0.0.23 d	108± 0.58 b	2.23± 0.044 c	2.4± 0.03 c	0.75± 0.029 c	0.78± 0.02 b
(Gp2) *Camallanus* Infested	104± 2.08 a	115.0± 2.89 a	178± 1.15 a	183± 1.15 a	3.98± 0.30 a	4.7± 0.06 a	1.35± 0.028 a	1.54± 0.07 a
(Gp3) Levamisole Treated	64± 0.58 c	41.00± 0.58 b	137± 0.57 c	110± 0.58 b	3.05± 0.029 b	2.47± 0.008 bc	0.92± 0.044 b	0.83± 0.015 b
(Gp4) Fenbendazol Treated	77± 0.57 b	43.67± 0.88 b	148± 1.15 b	110± 1.15 b	3.4± 0.057 b	2.58± 0.015 b	1.06± 0.065 b	0.89± 0.023 b

Different letters in the same column means significant difference at ($p \leq 0.05$). W= week PT= post treated

Discussion

Parasitological examination of catfish showed the *camallanus* species that have morphological features as red color, their location further toward the posterior of intestinal tract than other worm like parasites. Others characteristic features of genus *camallanus* also is smooth, cylindrical and short round worms, presence of both eggs and larvae within their bodies of worm (ovoviviparous)**.** Our observation agreed with **Levsen (2001).** Identification of genus *camallanus* by the body shape, form of mouth, lips, size of the buccal capsule, esophagus and tail shape these are characteristic features to easily recognized (**Mai 2018).** Same findings also confirmed by **Sahle *et al*., (2017)** who reported that the *camallanus* have indirect life cycle and fish is final definitive host. These referred to presence of both eggs and larvae within adult worm body of females (ovoviviparous) and presence of second vector as cyclops in water that carry 3rdstage (larvae) (**Moravec and Wang 2004).**

Chemical examination of water samples revealed not-significant change in water quality parameter. As these parameters were appeared within normal range of permissible levels when compared with normal reference of **FAO (2003) and (EPA, 2001)** as pure water at 25°C have pH 7. Same findings mentioned by **WHO (1993) and Osman *et al*., (2018).**

The clinical signs of infested catfish showed, off food, emaciation, deformed body shape and appearance of garish patches ulcers on dark color skin than normal. Death of some fish were showed in 2ndgroup. These may be due to *camallanus* nematodes infestation, which considered as one of the most harmful worm parasites deprive their host food and feed on host tissues, sera and blood causing emaciation **(Hassan *et al.* 2011).** The present observation agreed with **Leela and Rama (2014)** who seen the same clinical signs in infested fish with nematods that induced reduction in their growth, reproduction and survival.

The PM examinations of infested catfish showed paler gills, pericarditis, perihepatitis, stomatitis, enteritis, splenomegaly and nephritis. In addition, the gall bladder were enlarged and pale yellowish. Liver was enlarged and somewhat congested with petical hemorrhage. Spleen was enlarged. Kidneys were swollen, dark red and friable. Stomach was distended. The intestine were congested and had a watery yellowish mucous. These may be referred to *camallanus* caused serious injuries and inflammation to intestinal tract and various internal organs. Our observation coincided with **El-Bouhi and El-Qelsh (1993).** Same PM lesions also reported by **Adawy *et al*., (2012)** observed that the infested fish with helminthes induced congestion of the internal organs specially intestine and gills covered with slime and mucous on its surface. The counts of

white blood cell and differential leukocyte count are important parameter responsible for fish health status and applied with a measure of general immune response of fish **(Terrazas *et al*., 2011).**

The Leukogram in the infested group revealed a leukocytosis and eosinophilia. These may be reflection to *camallanus* infestation. Leukocytosis may be due to either neutralization of harmful effect of the parasite and adaptive nature to cope up for removing debris of damaged tissue or from the toxicant (endotoxins / exotoxins) secreted by parasites, **(Nnabuchi *et al*., 2015).** Same results are coincided with those reported by **Ruhela *et al*., (2012)** they recorded a significant increase in TLC in fish infested with parasites.

Significant increase in eosinophil (Eosinophilia**)** may be attributed to parasitic infestation, which caused hypersensitivity as eosinophils are play important role in defense mechanism, phagocytizing activities, inflammation and maintaining homeostasis (**Reite, 1998 and Murray *et al.* 2007).** Same results coincide with **Dezfuli and Giari (2008)** who reported eosinophilia during naturally infested fish and numerous released when antigens are continually being**.**

Monocytes and neutrophils levels are important white blood cells to protect the body through their phagocytic activities against parasitic infestation where it considered the first line of immune defense against pathogens overcoming the natural barriers and have ability to engulf and kill pathogens **(Ainsworth *et al*., 1991 and Sinha 2000).**

In our study, monocytes, heterophiles and lymphocytes were significantly decrease after exposure to *camallanus* infestation may be due to the stress and inhibitor of parasites in intestine led to migration and degranulation of cells to site of inflammation **(Rohlenová *et al*. 2011).** Our results agreed with **Adeyemo and Solomon (2014).**

A significant decrease in both phagocytic % and index at 1st and 2nd collection as compared to control group**.** These may be due to decrease in monocyte as a phagocytic cell in blood and macrophages in tissue. These led to immunosuppressive during parasitic infestation **(Secombes, 1996).** On the same manner, the phagocytes and other blood cells were migrates and colonized in tissues surrounding the attachment organs as stomach, intestine and gills during heavy parasitic infestation which induced decrease in phagocyte activity and inhibit host immune reaction **(Pellitero, 2008).** Our results are confirmed by histopathological lesions in the spleen as showed congestion, splenomegaly and haemosiderosis deposits in white and red pulps of 2ndgroup. Same results agreed with **Aly *et al*. (2011) and Osman *et al*., (2009)** as spleen showed vascular changes in catfish infested with trypanosome**.** The spleen size of fish is widely used as a simple measurable immune parameter, with a potential role in immune response against parasite infestation **(Roberts & Agius 2003).**

Concerning the serum analysis of proteins profile of infested catfish showed a significant decrease in total protein, albumin and globulin, A/G ratio when compared with control group. These alterations may be attributed to liver and kidney damage as demonstrated histo pathologically by perivascular aggregation of lymphocytes. Our results agreed with **El-Bouhi and El-Qelsh (1993) and Aly *et al* (2011)** who reported a significant decrease in protinogram, in Tilapia Nilotica infested with helminthes and catfish infected with *A. hydrophilic.* Parasitic infestation also causes disturbances in protein digestion in host and decreased plasma protein either due to loss by renal excretion as kidney showed renal tubular nephrosis and impaired protein and albumin synthesis by inflamed liver **(Leela and Rama 2014).** Beside loss of protein with hemorrhage from intestinal injures as harboring of *camallanus* spps through fish gut that resulting in malnutrition, mal-digestions and malabsorption. These were confirmed histopathologically as showed the larval stage of parasite in wall of intestine with hyperplasia of goblet cells and lymphocytic infiltration in lamina propria were recorded in all groups at the end of 4th weeks of experimental periods. Same results recorded by **Pahor*et al*., (2017)** they showed the intestine of the infested catfish exhibited separation of serosa and submucosa and deficiency of vitamins involving the digestive organ also increase protein catabolism due to stress from parasitic infestation. Similar results also agreement with

Ruhela *et al*., (2012) who observed inflammatory edema, a blunted tips of villi and separation in muscle layer with mononuclear macrophages conjunction with local tissue macrophages form epithelioid and multinucleated giant cells at site of intestinal inflammation led to anorexia, mal-digestions and malabsorption of infested catfish. Beside degenerative changes in hepatocytes and renal tubular epithelium that resulting in hypoproteinemia and hypoalbuminemia **(Roberts, 2012).**

C-reactive protein (CRP) is inflammatory marker (IM) for evaluating the health status of a herd, aquarium farms and provide us a diagnostic and a prognostic information about diseased condition **(Pathak and Agrawal, 2019**). C-reactive protein changed according to external or internal challenges such as parasitic infestation and considered non-specific innate immune component, play important roles in protection against parasitic infestation, clearance of damaged tissue, prevention of auto immunization and regulation of inflammatory response **(Mold *et al*., 2002).**

C-reactive protein (CRP) of infested group showed a significant increase at 1stand 2nd collection when compared with control group. These may be referred to the response of inflammatory stimuli/reaction of parasitic infestation, this increased include acute phase proteins (APP) and tend to remain elevated in chronic inflammatory condition (**Murata *et al*., 2004).** These results reinforced with **GÜLEÇ and CENGİZLER (2012)** who mentioned that CRP increased very rapidly in serum within 12- 24 hr. following tissue injury of any cause such as acute inflammation. Our results also agreed with **Pionnier *et al*., (2014).**

Humoral immune response in infested group revealed a significant increase in (IgM & IgD) when compared with control group. These may be attributed to *camallanus* infestation induce intestinal inflammation and tissue damage, and these results may be due to IgM & IgD considered effective in prevention and control of various fish diseases **(Harding *et al*., 1990).** The present results agreed with **Magnadottir, (1998)** who stated that the major component of fish specific humoral defense is IgM and IgD that increase in production against helminths. Same results also coincide with **Rohlenová *et al*. (2011)** who recorded that IgM increased at parasitic infestations where it specific antibodies play an essential role in cytotoxic mechanisms such as activation of complement system and helping leukocytes adhere to parasite surface through Fc-like receptors. Also IgD increased at infested diseases, as make cross-linking not only immune surveillance at interface between immunity and inflammation but also during tissue damage because of IgD bind to myeloid cells such as basophils and induce anti-microbial, ant-inflammatory and B-cell stimulating factors **(Chen and Cerutti, 2010)**.

The liver enzymes activities in infested group revealed a significant increase in AST and ALT when compared to control group**.** These alteration may be a reflect of hepatocellular damage as due to toxicant substances of metabolic products of nematodes infestation led to increased cellular permeability of hepatocytes and leakage of enzymes to serum**.** Similar results agreed with **Nnabuchi *et al*., (2015).** These alterations in our study were demonstrating histopathologically in liver by dilated portal tract with short fibrous septa extends to the hepatic parenchyma and congested portal vein with dilated and congested central vein with hemosiderin deposition, and lymphocytic infiltration**.** Our results coincided with **Ruhela *et al*. (2012)** who observed increase in serum enzyme levels of AST and ALT due to various toxic substances (exotoxins/endotoxins) secreted /excreted by the parasites harboring gut fish that caused necrosis of hepatocytes and damage of various tissues.

The infested catfish showed a significant increase in uric acid and creatinine levels when compared with control group. These may be attributed to kidney damage due to toxic substances of the parasites that caused nephrotoxicity. The main contributors to nephrotoxicity are the vasoactive amines and histamines produced due to antigen-antibody interaction during parasitic infestation **(Roberts, 2012)**. Our results agreed with **Holle *et al*. (2003)** who reported that the infested fish with helminthes caused insufficiency the gills and kidney function test led to increase uric acid and creatinine levels. These alterations in our study confirmed histopathologically as kidney showed

marked degeneration of epithelial lining renal tubules with mononuclear cells infiltrations. Our results are agreement with **El-Bouhi and El-Qelsh (1993**) **and Aly *et al*., (2011)** they observed inflammation of renal tubular of kidneys cells of Tilapia Nilotica infested with helminthes and catfish infected with *A. Hydrophilia*. **Gills** of infested non treated catfish with *camallanus* spps histopathologically shown degeneration of primary lamellae with absence of secondary lamellae at the end of 4 weeks. These alterations confirmed by those showed with **Ruhela *et al*., (2012)** who reported a significant increase in uric acid and creatinine levels attributed to increase protein catabolism where tissue damage, oxidative stress from parasitic infestation and environmental stress. Dysfunction of kidney and gills also microscopically showed degeneration of primary and secondary gill lamellae due to parasitic infestation of catfish **(Dezfuli and Giari 2008).**

Treated by levamisole and fenbendazole in infested groups revealed improvement in the clinical signs, hematological, immuno-biochemical parameters and pathological changes, which returned to within normal levels in (Gp3 & 4) at 2ndcollection when compared to control group after 4th weeks post treatment. These due to anti-helminthic effect and nematocidal activity of levamisole and fenbendazole in cleared the *camallanus spps* worms in catfish. The present results supported by studies of **El-Bouhi and El-Qelsh (1993) and Aly *et al*., (2011)** they reported that the treated with levamisole and fenbendazole ameliorates adverse effects of gastrointestinal nematode as *camallanus* spps infested catfish. Where it's making complete clearance of *camallanus* worms and its larvae, so enhance appetite of fish and immune system stimulator, additional to it improve weight gain **(Pahor *et al*., 2017)**. Beside the fast effect of fenbendazole on worm and protruded it through out anus in 24 hr **(Noga, 2010)**. Furthermore, **Brengun**, **(2009)** reported that levamisole is the most drug use in riding nematodes as it great fast acting de-wormer and is highly recommended for treatment of internal parasites among fish. Levamisole is more effective than fenbendazole due to restores and enhance immune response as stimulation immunoglobulins forming cells **(Baba *et al*., 1993)**. Also, it raising phagocytosis as increased phagocytic activity (% & index) and cell-mediated immune reactivity, attributed to it potent rate of T -lymphocytes differentiation and responsiveness to antigens and mitogens. Additionally to enhance effect of non-specific immune response as increasing function of macrophages chemotaxis which antigen trapping and processing that indicated levamisole is immune stimulant beside immunomodulatory effect **(Siwicki *et al*., 1990 and Shah *et al*., 2011).** Worms are excreted within 24 hr. by levamisole, while fenbendazole take less than 6 hr. for the worms start excreted out the fish, these due to both above medications makes paralysis to worms and forcing them to release from their hold on the intestinal walls and then passed out of fish body **(Discussion, 2013).**

Conclusion

Finally, it could be concluded that the treatment with levamisole and fenbendazole were effective against naturally infestation with *camallanus* spps in catfish (*clarias gariepenus*) where ameliorating clinical signs, hematological, immuno-biochemical parameters and pathological changes. In addition to, levamisole is more effective than fenbendazole in enhancing the immune response of catfish against *camallanus* spps worm's as nematodes parasitic infestation.

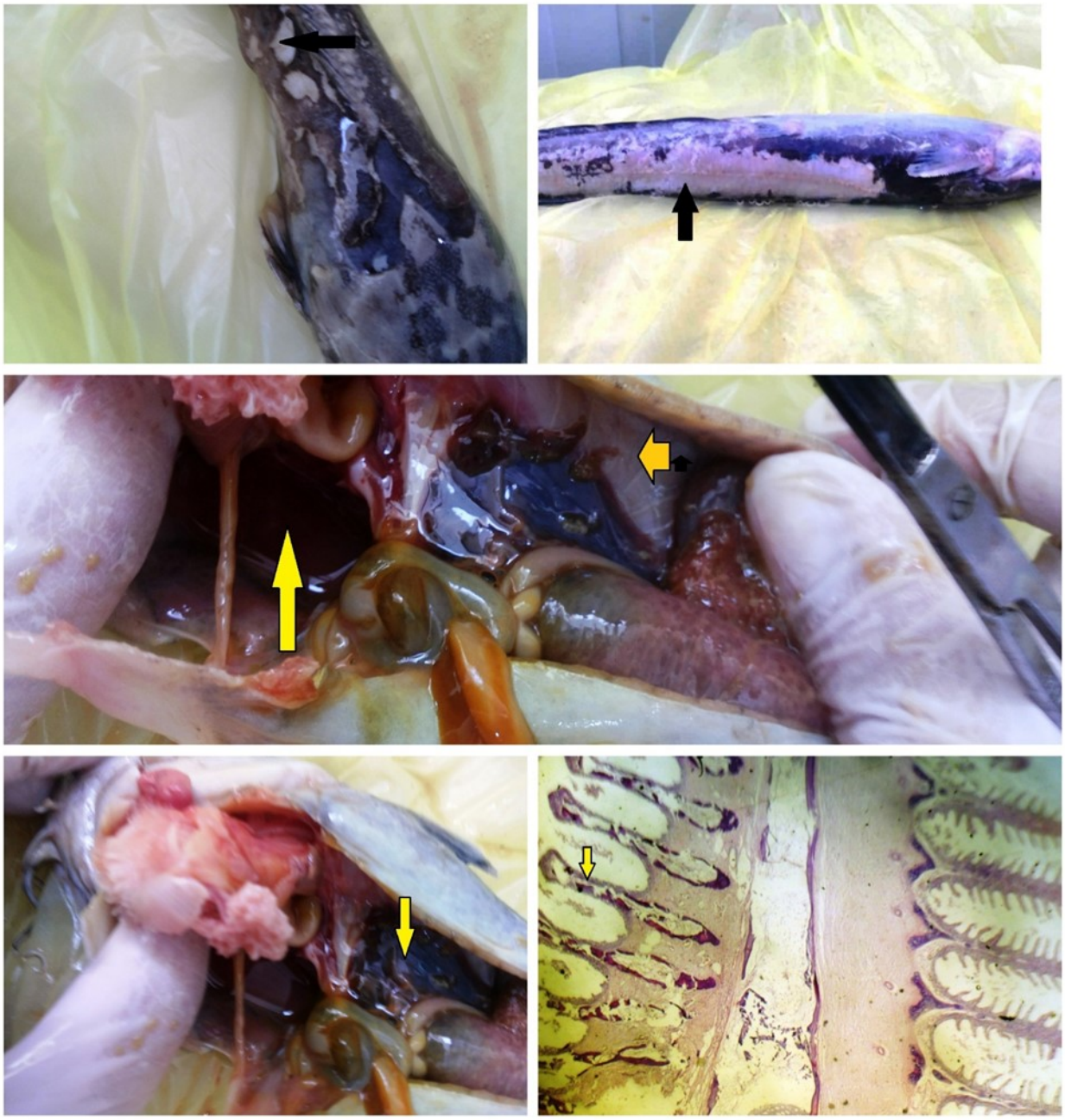

Figure (1): A & B : Catfish infested with *camallanus* showed ulceration of skin. **C:** Catfish infested with *camallanus* showed congestion of internal organs. **D:** Catfish infested with camallanous showed pale gills, congestion of internal organs. **E:** Photomicrograph of gills of (Gp2) infested non-treated catfish with *camallanus* showing degeneration of primary lamellae with absence of secondary lamellae (H& E x 200).

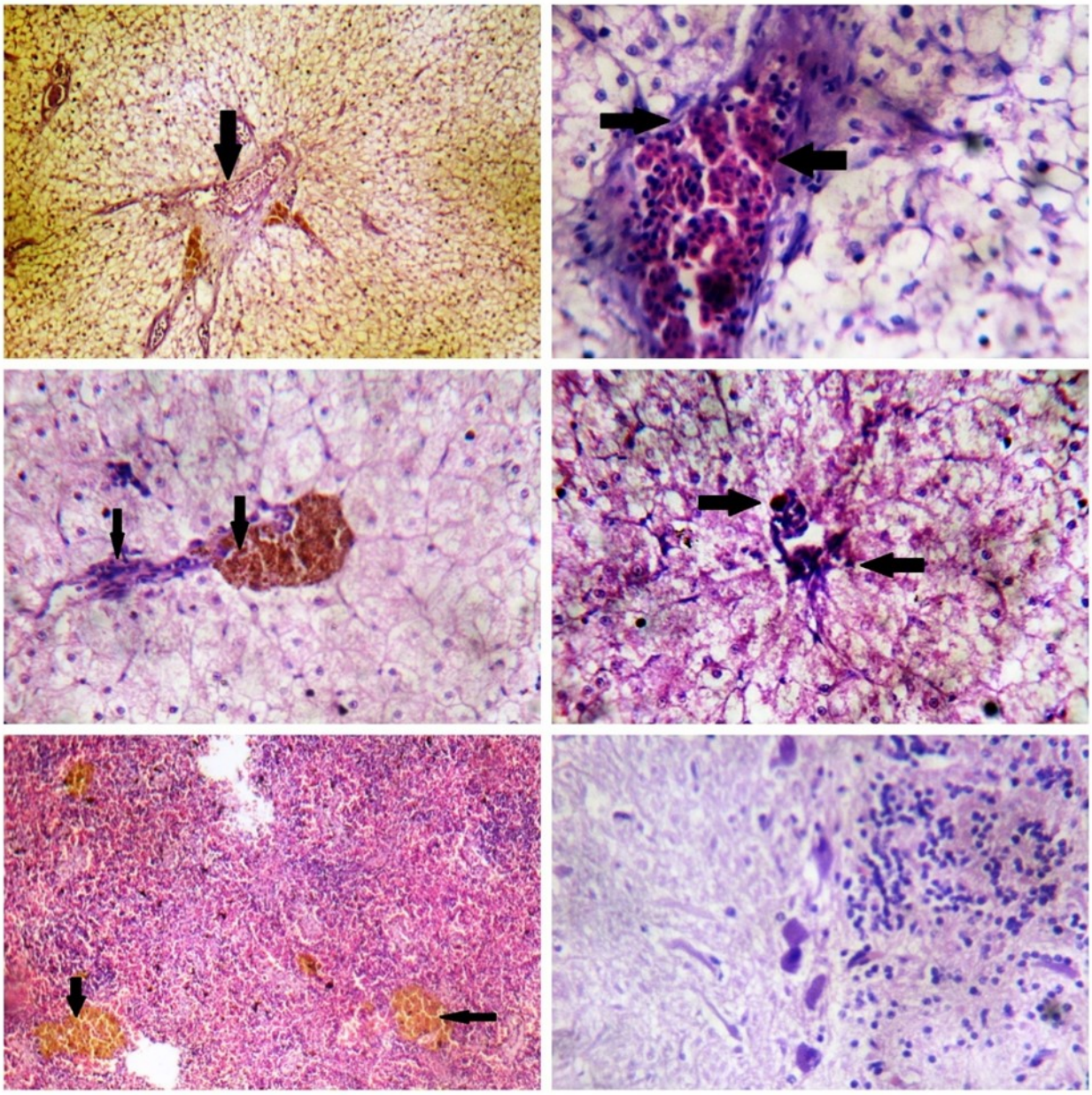

Figure (2): A: Photomicrograph of liver of (Gp2) infested non-treated catfish with *camallanus* showing dilated portal tract with short fibrous septa extends to the hepatic parenchyma and congested portal vein (H& E x 200). **B:** Photomicrograph of liver of (Gp2) infested non-treated catfish with *camallanus* showing dilated portal area with inflammatory cells (H& E x 400). **C:** Photomicrograph of liver of (Gp3) infested catfish with *camallanus* and trated with levamisole showing dilated and congested central vein and thin fibrous band extend to the parenchyma infiltrated by mild lymphocytic infiltrate (H& E x 400). **D:** Photomicrograph of liver of (Gp4) infested catfish with *camallanus* and treated with fenbendizole showing mildly dilated central vein surrounded by mildly degenerated hepatocytes (H& E x 200). **E:** Photomicrograph of spleen of (Gp2) infested non-treated catfish with *camallanus* showing congestion in white and red pulps. (H& E x 200). **F:** Photomicrograph of Kidneys of group 4 infested catfish with *camallanus* and treated with albendizole showing characteristic molecular layer ,purkinje cell layer (swollen purkinje cells) and granular layer (H& E x 200).

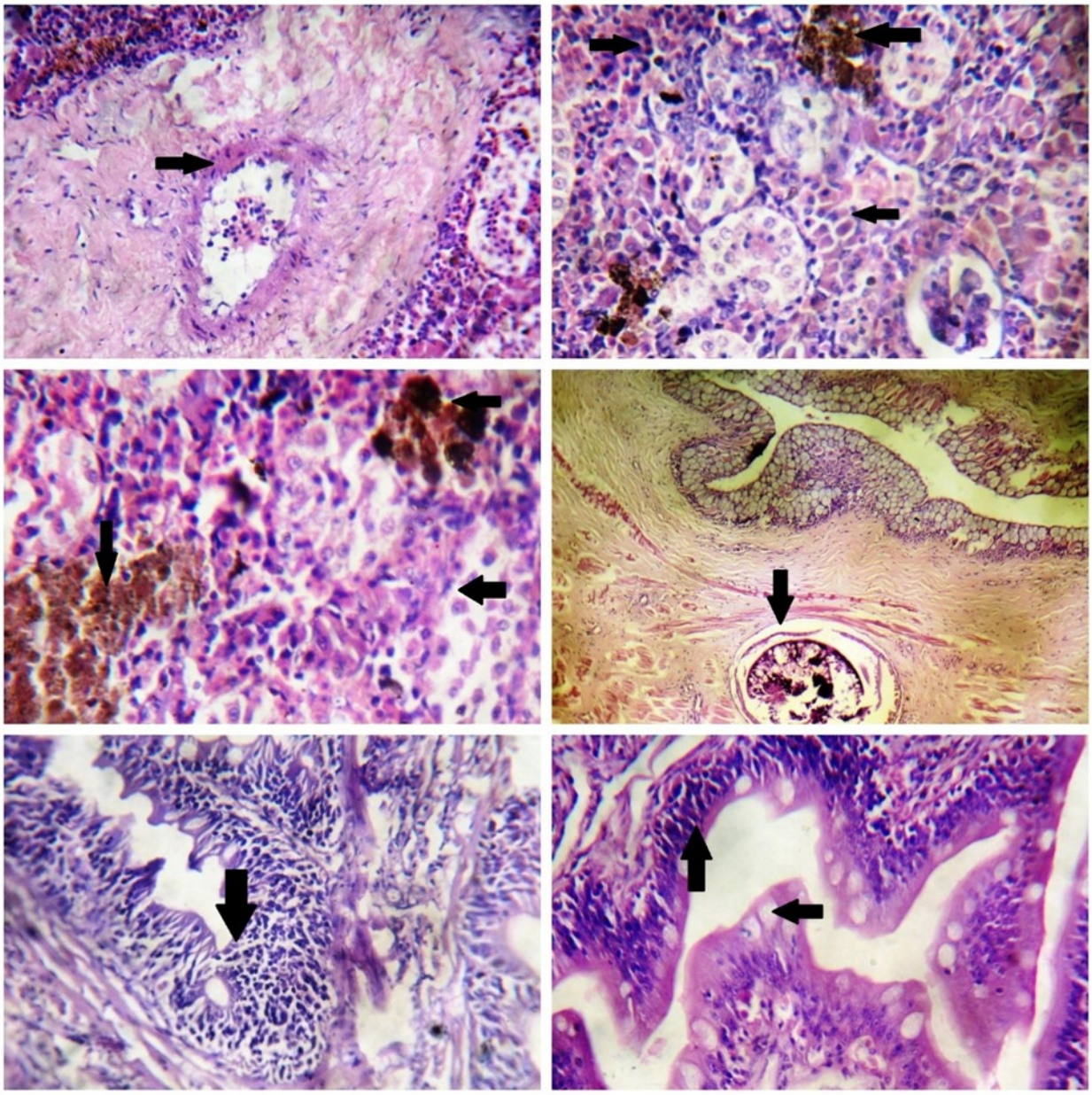

Figure (3): A: Photomicrograph of kidneys of (Gp2) infested non-treated catfish with *camallanus* showing thickened renal blood vessel with narrow lumen surrounded by mononuclear inflammatory cells and degenerated renal tubules (H&Ex200). **B:** Photomicrograph of kidneys of (Gp2) infested non treated catfish with *camallanus* showing heavy stromal mononuclear inflammatory cellular infiltrates, heavy hemosiderin deposition and degenerated renal tubules (H& E x 400). **C:** Photomicrograph of kidneys of (Gp2) infested catfish with *camallanus* and treated with fenbendazole showing heavy stromal hemosiderin deposition ,hemosiderin laden macrophages,and markedly degenerated renal tubules (H& E x 400). **D:** Photomicrograph of intestine of (Gp2) infested non-treated catfish with *camallanus* showing larval parasite in the wall of the stomach (H& E x 400). **E:** Photomicrograph of Intestine of (Gp3) infested catfish with *camallanus* and treated with levamisole showing hyperplasia of the goblet cells and lymphocytic infiltration in lamina propria (H& E x 400). **F:** Photomicrograph of intestine of (Gp4) infested catfish with *camallanus* and treated with fenbendazole showing hyperplasia of the goblet cells and lymphocytic infiltration in lamina propria (H& E x 400).

References

Abdel-Gaber, R.; El Garhy, M. and Morsy, K. (2015). Prevalence and Intensity of Helminth Parasites of African Catfish *Clarias gariepinus* in Lake Manzala, Egypt. *Advances in Bioscience and Biotechnology*. *6*(07), 464.

Adawy, R.S.; Shalaby, H.A. and Deeb, K.A. (2012). Clinico Pathological Studies on the effect of Nematode and Cystode infections on trace elements concentration in (Clarias gariepenus in Dakahlia Province. Egypt. J. Agric. Res. 90 (1): 423-439.

Adeyemo, B.T. and Solomon, S.G. (2014). Hematological reference ranges of cultured Clarias gariepinaaus in the Lower Benue River Basin, Nigeria. Haematological and serum biochemical reference ranges of the fresh water fish. turtle PE lomedusa: Article *in* Comparative Clinical Pathology March University of Abuja.

Aly, S.M.; Abdallah, O.; Mahmoud, A. and Gafer, H. (2011). Efficiency of Levamisole in Improving the Immune Response of Catfish (Clarias gariepenus) to Aeromonas hydrophila Vaccine: Clinico-Pathological Studies.Mediterranean Aquaculture J. 1(1); 8-17. 4(1); 18-26.Original Article.

Ainsworth, A.; Dexiang, C.; Water, P. and Greenway, T. (1991). Effect of temperature on the immune system of channel catfish (Ictalurus punctatus). I. Leucocyte distribution and phagocyte function in the anterior kidney at 10°C. Comp Biochem Phys A.100: 907-912.

Anthony, T.W.; Twin, K.M.; Erin, M.W. and Michael, E.M. (1985). "Phagocytic and killing capacities of utrine derived leukocytes. From mares, resistance and susceptible to chronic endometritis". Am. J. Vet. Res. V. 46, N (9) P: 1938- 1940.

Anthony, E.O.; Christopher, E.O. and Blessing, J.O. (2014). Gastrointestinal Helminth Parasites Community of Fish Species in a Niger Delta Tidal Creek, Nigeria. Journal of Ecosystems. http://dx. doi. org/ 10. 1155/2014/246283. Department of Animal and Environmental Biology, University of Benin, Benin City 1154, Nigeria. Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo, Uyo, Akwa IbomState 1017, Nigeria.

Baba, T.; Watase, Y. and Yoshinaga, Y. (1993). Activation of mononuclear phagocyte function by levamisole immersion in carp. Nippon Suisan Gakkaishi. 59, pp. 301-307.

Booth, N.H. and McDonald, C.E. (1982). Veterinary pharmacology and therapeutics. 5th ed. Iowa State University Press, Ames, Iowa.

Boyum, A. (1986). "Isolation of mononuclear cells and granulocytes from human blood". Canadian. J. Clin. Invest. V. 21, P: 77-89.

Brengun, B. (2009). How to Treat Camallanus Worms.Article http://.com/article...? e=321&catid=2**.**Article.http://www.loaches. com/Members/shari...ydrochloride-1**.** PlecoPlanet Forum Rules. Last edited by bre; 22-04-09 at 01:22. Location: Burrum Heads, Queens land, Australia. Breeding Experience: Intermediate. Posts: 5,030. Gallery: 161. Helpful Posts. Awards.

Chandy, M.L.; Cristalle, S.; Sreeja, P.; Kumar, S.K. and Renju, J. (2016). Understanding molecular mechanisms in multivariant actions of levamisole as an anti-helminthic, anti-inflammatory, antioxidant, anti-neoplastic and immunomodulatory drug. G Model JOMSMP; 494: 4.

Chen, K. and Cerutti, A. (2010). Immunological Reviews: New insights into the Enigma of Immunoglobulin D. 237 (1):160.

Chu, Y. and Dietert, R.R. (1989). "Monocytes function in chicken with hereditary dystrophy". Poult. Sci. V, 68 P: 226-232.

Dayan, A. (2003). Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. Acta Trop. 86: 141-159.

De, N.C. and Maity, R.N. (2000). Development of *Procamallanussaccobranchi* (Nematoda: Camallanidae), a parasite of afreshwater fish in India. *Folia. Parasitol*. **47**, 216-226.

Dezfuli, B.S. and Giari, L. (2008). Mast cells in the gills and intestines of naturally infected fish: evidence of migration and degranulation. *J. Fish Dis*.**31,** 845-52.

Diana, Walstad (2017). Treating Fish for CAMALLANUS and Other NEMATODES. P: 1-7 For more information about her books, see: http://dianawalstad.com. 20.

Discussion (2013). at UK Aquatic Plant ociet.https://www.ukaps.org/forum/threads/levamisole-hydrochloride-shrimp-and-water-clarity.28000/.

Doumas, B.; Carter, R.; Peers, T. and Schaffer, R. (1981). "Method for determination of totalprotein in serum". *Clin. Chem*. 27. 1642.

Drupt, F. (1974). "Depression of human serum albumin". *Farm. Boil*.V.9, P: 222-229.

Edward, J. N. (1999). Fish disease: Diagnosis and treatment. Iowa State: Black well. p. 170.

(EPA) Environmental Protection Agency (2001). Nitrate/Nitrite-N in Water and Bio solids by Automated Photometry. Office of Water Office of Science and Technology Engineering and Analysis Division (4303) 1200 Pennsylvania Ave., NW Washington, DC 20460 U.S. Method 1685. 821-R-01-016.

El-Bouhi, Z.M. and El-Qelsh, M.E. (1993). Evaluation of the anthelmintic efficacy of levamisole and its side effects on Tilapia nilotica (Oreochromus niloticus). Zagazig Veterinary Journal. 21 (3), pp. 497-511.

Erhard, M.; Vonquistrpl, Schrmnr, Kasprs B. and Kinlmann, R. (1992). Development of specific enzyme linked immunosorbentantibody assay for detection immunoglobulins. using monoclonal antibodies. Poltry. Sci. 71, 302– 310.

FAO, (2003). Fisheries and Aquaculture Country Profile. Available at ftp://ftp.fao.org/FI/DOCUMENT/fcp/en/FI_CP_ET.pdf.

Godeeris, B.M.; Boldwin, C.L.; Ole-Moiyoio and Morrison, W.I. (1986). Improved methods for purification and depletion of monocytes from bovine peripheral blood mononuclear cells. Functional evaluation of monocytes in response to Lectins". *Imm. Meth. J.*V. 89, N (2) P: 165- 73.

GÜLEÇ, A.K. and CENGİZLER, İ. (2012). Determination of acute phase proteins after experimental *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus* L.). Turk. J. Vet. Anim. Sci. 36(4): 380-387.

Hassan, A.A.; Akinsanya, B. and Adegbaju, W.A. (2011). Impacts of Helminth Parasites on *Clarias gariepinus* and *Synodontis clarias* from Lekki Lagoon, Lagos, Nigeria. *Report and Opinion*, **2**, 42-48.

Harding, F.A.; Amemiya, C.T.; Litman, R.T.; Cohen, N. and Litman, G.W. (1990). Two distinct immunoglobulin heavy chain isotypes in a primitive, cartilaginous fish, Rajaerinacea. Nucleic Acids Res, 18: 6369-6376.

Henry, R.Z. (1974). "Clinical chemistry principle and Techniques". 2nd Ed, *Harper and Raw*, p. 52.

Hoffman, G.L. (2019). Parasites of North American freshwater fishs. Cornell University Press.

Holle, D.; Lewis, J.; Schuwerack, P.; Chakravarthy, C.; Shrive, A.; Greenhough, T. and Cartwright, J. (2003). Inflammatory interactions in fish exposed to pollutants and parasites: a role for apoptosis and C reactive protein. *Parasitology* **126**, S71-S85.

Jain, N.C. (1993). Essentials Of Veterinary Hematology. Lea & Fibiger. Philadelphia. USA.

Leela, B. and Rama, R.K. (2014). Nematode Parasites in a Freshwater Fish *Glossogo biusgiuris* (Hamilton-Buchanan, 1822) at Lower Manair Dam, Karimnagar Dt. Andhra Pradesh, India. Department of Zoology Government Degree College, Jammikunta, Satavahana University, Karimnagar Dt. Andhra Pradesh India. *IOSR Journal of Pharmacy and BiologicalSciences (IOSR-JPBS),* 9(2): 37-40.

Levsen, A. (2001). Transmission ecology and larval behavior of *Camallanus cotti* (Nematoda, Camallanidae) under aquarium conditions. *Aquarium Sci. Conservation* 3: 301-311.

Lucky, Z. (1977). Methods for diagnosis of fish diseases. Amerind publishing Co. New Delhi, India.

Mai, W. (2018). Plant-parasitic nematodes: a pictorial key to genera. Cornell University Press.

Magnadottir, B. (1998). Comparison of immunoglobulin (IgM) from four fish species. BÚVÍSINDI. ICEL. AGR. SCI. 12: 47–59. Institute for Experimental Pathology, University of Iceland, Keldur, IS-112 Reykjavík,

Iceland(e-mail: <bergmagn@rhi.hi.is>).

Marie, A. Harbin and Van den Berg , C.M.G. (1993). Determination of ammonia in seawater using catalytic cathodic stripping voltammetry. Analytical chemistry. 65. (2-3), 3411-3416. Dol: 10.1021/ac 000 71a 013.

Mold, C.; Rodriquez, W. and Rodic, P.B. (2002). C-reactive protein mediates protection from lipopolysaccharide through interactions with fc- gamma. immun. 169:7019-7025.

Moravec, F.; Nie, P. and Wang, G. (2004c) . Reduction of *Camallanus hypothalmichthys* Dogel and Akhmerov, 1959 (Nematoda: Camallanidae) and its first record from fishes in China. *J. Parasitol*.90:1463-1467.

Murata, H.; Hsimada, N. and Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. The Vet. J. 168: 28-40.

Murray, H.M.; Leggiadro, C.T. and Douglas, S.E. (2007). Immuno-cytochemical localization of leucocidin to thecytoplasmic granules of eosinophilic granular cells from thewinter flounder gill. *J. Fish Biol.***70,** 336 – 45.

Nathan, B.R. and Scheld, W.M. (2002). The potential roles of C-reactive protein and procalcitonin concentrations in the serum and cerebrospinal fluid in the diagnosis of bacterial meningitis, Curr Clin Top Infect Dis. 22: 155-165.(Biology).

Nnabuchi, U.O.; Ejikeme, O.G.; Didiugwu, N.C.; Ncha, O.S.; Onahs, S.P. and Amarachi, A. (2015). Effect of parasites on the biochemical and haematological indices of some clariid (Siluriformes) catfishes from Anambra River, Nigeria. Intern. J. of Fisheries and Aquatic Studies; 3(2): 331-336

Noga, E.J. (2010). Fish Disease: Diagnosis and Treatment. Wiley-Blackwell (Hoboken, NJ).P: 536.

Okoye, U.O.; Ndupuh, E.E. and Adeleye, S.A. (2016). A survey on endo-parasites of *Clarias gariepinus* in some selected fish farms in Owerri west local government area of Imo state, Nigeria.

Osman, H.A.; Fadel, N.G. and Ali, A.T. (2009). Biochemical and histopathological alterations in catfish, Clariasgariepinus infected with trypanosomiasis with special reference to immunization. Egypt. J. Comp. Path. & Clinic. Path. Vol. 22 No. 3 (July); 164 – 181.

Osman, A.G.M.; AbouelFadl, K.Y.; Abd El Reheem, A.M.; Mahmoud, U.M.; Kloas, W. and Moustafa, M.A. (2018). Blood Biomarkers in Nile tilapia *Oreochromis niloticus niloticus* and African Catfish *Clarias gariepinus* to Evaluate Water Quality of the River Nile. Journal of Fisheries Sciences**.**12 (1): 001-010.

Reite, O.B. (1998). Mast cells/eosinophilic granule cells of teleostean fish: a review focusing on staining properties and functional responses. Fish Shellfish Immunol.**8,** 489 – 513..

Reitman, S. and Frankel, S. (1957). "Calorimetric determination of S Got and S Gpt activity". Am. J. Clin. Path. N (28) P: 56 – 59.

Roberts, R.J. (2012). Fish Pathology.4thED**.** BVMS, PhD .Blackwell Publishing

Roberts, R.J. and Agius, C. (2003). Melanomacrophage centers and their role in fish pathology, *J. Fish Dis.* **26**, 499 – 509.

Rohlenová, K.; Morand, S.; Hyršl, P.; Tolarová, S.; Flajšhans, M. and Šimková, A. (2011). Are fish immune systems really affected by parasites? an immunoecological study of Common carp (Cyprinus carpio). Parasites & Vectors, 4:120. P 2:18

Ruhela, S.; Pandey, A.K. and Khare, A.K. (2012). Hematological, Biochemical and Histopathological Changes in Clarias Batrachus (LINN.) Induced By experimental *Procamallanus*. Infection. Biochem. Cell. Arch Vol. 12, No. 1, pp. 189-204.

Pahor-Filho, E.; Castillo, A.; Pereira, N.; Pilarski, F. and Urbinati, E. (2017). Levamisole enhances the innate immune response and prevents increased cortisol levels in stressed pacu (Piaractus mesopotamicus). Fish & Shellfish Immunology. 65: 96-102.

Pathak, A. and Agrawal, A. (2019). Evolution of C- reactive protein. Frontiers in Immunology. | www.frontiers in.org. V. 10 | Article 943. P: 1-12.

Pellitero, A.P. (2008). Fish immunity and parasite infections: from innate immunity to immune prophylactic prospects. Vet Immunol Immunop. 126: 171-198.

Pionnier, N.; Adamek, M. and Hoole, D. (2014). C-reactive protein and complement

as acut phase reactants in common carp Cyprinus carpio during CyH V- 3 infection. Diseases of aquatic organisms.

Sahle, H.; Enbiyale, G.; Agonafir, H.; Mitiku, F. and Neges, T. (2017). Review On *Capillaria* And *Camallanus* Nematode Parasites Of Fresh Water Fish In Ethiopia. J. Am. Sci. 13(8): 85-92.

Sanders, G.T.B.; Pas man, A.J. and Hoek, F.J. (1980). "Determination of seru8-m uric acid". Clin. Chem. Acta. V. 101, P: 299- 303.

Scott, W.E.P. and Pamela, G. (2015). Parasitology and Necropsy of Fish. Sensors. (7), 875-881.DOL:10.1021 Compendium Vet.com | February 2009 North Carolina State University 9 PUBLICATIONS 98 CITATIONS SEE PROFILE

Secombes, C.J. (1996). The nonspecific immune system: Cellular defence. In The Fish Immune System. Organism, Pathogen and Environment. Edited by: Iwama G, Nakanishi T. San Diego: Academic Press; pp. 63-103.

Schludermann, C.; Konecny, R.; Laimgruber, S. and Auteurs, J.W. (2003). Fish Macroscopic Parasites as Indicator of Heavy Metal Pollution in River Sites in Austria. *Parasitolo*.30, 201-238.

Shah, D.; Londhe, V.; Mazumder, R. and Parikh, R. (2011). Can levamisole alone maintain the immunity? International J. of Pharmacy and Pharmaceutical Sci.3:161-4.

Sinha, K.P. (2000). Hematological manifestation in *Clarias batrachus* carrying helminth infections. *J. Parasit. Dis*. 24, 167-170.

SPSS 14 (2006). Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June. Standard Version, Copyright SPSS Inc., 1989, 2006 All Rights Reserved, Copyright ® SPSS Inc.

Survarna, K.; Lyton, C. and Bancroft, J. (2013). "Bancroft's theory and practice of histological Techniques. 7 th Ed. Churchil Livingston. Elsevier.

Siwicki, A.K.; Anderson, D.P. and Dixon, O.W. (1990). In vitro immune stimulation of rainbow trout (Oncorhynchus mykiss) spleen cells with levamisole. Developmental and Comparative Immunology. 14 (2), pp. 231-237.

Terrazas, L.I.; Satoskar, A.R.; Montor, J.M. and Sosa, M.R. (2011). Innate and Cellular Immunology in Parasitic Diseases. Editorial. Int. J. Biol. Sci. tertilrlfiliclcieces. 7(9):1216-1219.

Tietz, N. (1996). Liver function tests, nitrogen metabolites and renal function In: Fundamentals of Clinical Chemistry 3rd ed. W. B. Saunders, Philadelphia. P: 476-576.

Wilikinson, P.C. (1976). "Recognition and response in mononuclear and granular phagocytes".Clin. Exp. Immunol, 25 (3): 355-366.

WHO (1993). guide lines for drinking water quality 2nd Ed. Vol. (1). Recommendations, WHO, Geneva.

Yanong, R.P.E. (2011). Tropical Aquaculture Laboratory, Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension - Service, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, FL 33570-3434. This document is Circular 91, one of a series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. First Published: December 2002. Major revision: August 2006. Reviewed June 2011.

Zhokhov, A.E. and Mironovsky (2007). Methods of the Complete Parasitological Dissection of Fish. Moscow, Addis Ababa, p. 8.

Zhang, H.; Kang, S.; Wang, G.; Zhang, Y. and Zhaa (2016). Fluorescence determination of nitrite in water using prown-shell derived Nitrogen-Doped carbon Nanodots as Fluorophores. ACS.