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Impact of applying Albendazole and Levamisole against some intestinal parasites in fish

By

Rehab E. M. Mowafy¹, Hend M. Megahed², Sarah A. A. Ibrahim³, Sanaa M. salim⁴, Abd-Allah S. Hamed⁵, Hala Salah⁶ and Soad Mekawy⁷

Pathology and Clinical Path. Dep.^(1,4,5&7) Fish disease Dep.⁽³⁾ and Biochemical Dep.^(2&6), Zagazig Provincial Lab. AHRI, ARC

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ABSTRACT

The continuous search for more effective therapeutic strategies and drugs to overcome the fishes' intestinal parasites was an incentive to carry out this study in the context of drug resistance and because of parasitic diversity in both *Oreochromis niloticus* and *Clarias garpeinus* leading to economic problems. Two stages have been implemented to evaluate the therapeutic effect of the most used anthelmintic drugs albendazole, and levamisole. The survey of some fish farms for internal parasites was applied on 320 fish as a first stage. The parasitological examinations were applied on fish fecal matter including physical examination and clinical signs. pathological investigation in companion with parasitological one was executed on 50 positive fish obtained from positive cases previously examined. Sixty-six infected fish from first stage were transported to the second stage which is experimental by divided it into groups compared with non-infected fish. Full pathological, hematological and biochemical studies on experimental fish were executed. Our results of first stage were exhibited total-ly 116 positive cases with prevalence (116/320) 36.25% of infestation with intestinal parasites. Five helminth parasites represented by cestode, nematode and trematode were isolated from total 50 dissected fish. The results of second stage were in favor of the treated groups showed marked improvement while both antiparasitic drugs revealed nearly similar results. Full pathological, hematological and biochemical studies were introduced.

INTRODUCTION

More than one and a half billion people worldwide depend on fish to supply 20% of the

total average animal protein intake. Fish farming is due to the increase in the need of animal protein in tropical areas (Areda, et al. 2019).

*Corresponding author: Rehab E. M. Mowafy, Zagazig Provincial Lab., Animal Health Research Institute, Agricultural Research Center, Giza, Egypt
E-mail address: mowafyrehab@yahoo.com
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Egyptian expansion in fish aquaculture requires improvement in fish health management, (Al Malki , 2021). Knowledge of endemic fauna of helminths in fish is of great importance, parasites act as stressors to the health of their hosts, (Radwan et al. 2021). Parasitic infection in fish is prevalent all over the world and of particular importance in tropical areas (Soliman & Nasr, 2015). Parasitic infection in fish refers to disease conditions resulting from organisms living in the fish causing significant reduction in the production sector (Bassey, 2011). They make a decline in growth rate, weight loss, affect fish production, and increase fish mortalities (Chandra, 2006).

Fish is infected by different parasites and can act as intermediate hosts for many digenesis and cestodes, but they also can act as definitive hosts for many helminths. (Feist and Longshaw, 2008). The most common parasites of fish are gastrointestinal parasites that compete with the fish host for nutrients, hence reducing the essential nutrients to be absorbed by fish. Subsequently, these parasites decrease the growth of fish leading to morbidity and mortality and making the fish more susceptible to surrounding predators (Azadikhah et al. 2014 and Omeji et al. 2015).

Clarias gariepinus has been considered as an important fish for farming in Africa. *C. gariepinus* has many advantages such as, having a wide range of geographical distribution, high growth rate, not affected with handling and trauma, and well appreciated in many African countries (Akinsanya and Otubanjo, 2006). Nile tilapia (*Oreochromis niloticus*) is the most common fish currently being, cultured commercially (Abdel-Tawwab and El-Marakby , 2004). In intensive fish farming, managing and controlling helminthic infestation have a constant challenge, since these tasks are complicated by the limited availability of licensed anthelmintic drugs (Zuskova et al. 2018) with varying depresses of effectiveness. Anthelmintic drugs albendazole, and levamisole present a low toxicity (Hirazawa et al. 2013; Martins et al. 2001).

Histopathology is a key for identification the parasitic infection target organ and mode of

action at the organization (disease severity), which affect ecosystem function at the organization population level (Schwaiger , 2001).

Applying histopathological tools consider one of the greatest advantages in environmental screening, this category allows examining specific target organs including intestine, kidney and liver Furthermore, the pathological alterations found in these organs are normally easier to identify than functional ones (Fanta et al. 2003).

The objective of this study was to determine ameliorative efficacy of some anti-parasitic drugs control fish intestinal parasites and study prevalence rate of these parasites on *Oreochromis niloticus* and *Clarias gariepinus* fish with regarded to pathological, hematological and biochemical assessment.

MATERIAL and METHODS

First stage

The present study was carried out in July 2022 on some aquaculture. 320 lived fish were examined through fecal content and physical examination for intestinal parasites from 8 fish farms located in Abbassa, Al-hosania and Faqoos cities being in Sharkia Governorate, 160 fish from each species (20 *Oreochromis niloticus* / farm and 20 *Clarias gariepinus* / farm) were examined. Fifty infected fish from 320 were dissected and examined for parasitological examinations involved the skin, fins, gills, alimentary tract and body cavity of fish as a first stage. Samples were left for a few minutes into a petri dish containing saline solution, then opened and examined by light microscope. Trematodes and cestodes were preserved in 10% formalin for fixation, while nematodes were fixed by using ethyl alcohol (70%) directly after collection, (Marcogliese, 2001) and prepared for histopathological examination parasites were identified by light-microscope using the standard keys in the literature, (Bray et al. 2005) for trematodes, and (Bunkley & Williams, 1996; and Vernon, 2006) for cestodes and nematodes, respectively.

Fecal examination and egg count

Wisconsin sugar flotation fecal egg count technique

To perform this technique place five grams of feces into a plastic cup, then add 10 ml of Shteather's solution (made by adding 454 grams sugar to 355 ml of hot water and stirring until dissolved) then cool in the refrigerator) Mix the sample then place a funnel into a 15 ml centrifuge tube.

Centrifuge the test tube for 2-4 minutes, then fill the tube with the sugar solution, then place coverslip and let it for 5 minutes on a slide. Count all eggs using the 10x on the microscope. Once all of the eggs are counted, take the number and divided by 3 to determine the eggs per gram of feces (Bliss and Kvasnick , 1997)

Second stage

Experimental study :

Sixty six infected lived fish from both *Oreochromis niloticus* and *Clarias garipinus* were transported from first stage immediately into experimental tanks in (Aquaculture Diseases Research unit of Zagazig provincial lab, Animal Health Research Institute). Infected fish were divided into three groups / species compared with control group as following

- Gp.1 :Control non infected fish
- Gp.2 :Infected non treated
- Gp.3: Infected fish and treated with Albendazole
- Gp.4: Infected fish and treated with Levamisole

Samples either blood or tissues for different hematological, biochemical and histopathological examinations were collected after 72 hours from drug applying. Designated groups were: Full designated protocol of both stages was illustrated in figure (1)

Experimental drugs:

Levamisole (Levamisole hydrochloride) ®: produced by (Sigma chemical company) bath 125 mg/L for 24 h.
Albendazole (Albendazole 2.5%) ® produced

by Pharma Swede company and used in form of bath at dose of 500 mg/L for 24 h. Both Levamisole and Albendazole according to Dina Walstad, (2017)

Blood samples:

Two types of pooled blood samples were collected from each farm from caudal vein under complete a septic condition. The first blood sample was collected on EDTA for hematological examination (1ml). The second blood sample was taken without anticoagulant in a clean and dry centrifuge tube (3 ml), left to clot at room temperature and centrifuged at 3000 rpm for 5 min. Serum was collected, labeled, placed in dry clean-capped tubes and frozen at -20°C for biochemical analysis.

The hematological and biochemical study:

The erythrocytic count was carried out according to Feldman et al. (2000), hemoglobin concentration, packed cell volume and total leucocytic count were carried out according to Blaxhall and Daisley (1973). Differential leucocytic count were calculated according to (Cole, 1986). Superoxide dismutase (SOD) was assayed according to Kakkar et al. (1984). And total protein was assayed according to (Doumas et al. 1981) albumin (Drupt ,1974), Malondialdehyde (MDA) was assayed according to Satoh (1987) using chemical kits (Biomed Egypt). Also lyophilized *Micrococcus lysodekcticus* used for serum lysozomal activity (Sigma M 3770).

Statistical analysis:

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range (Duncan, 1955) was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SPSS program SPSS (2004).

Pathological examination

Specimens from infected fish in both stages from affected organs as liver, intestine, stomach in addition to skin and gills were collected from dissected *Oreochromis niloticus*

and *Clarias gariepinus* and fixed in 10% buffered neutral formalin. Paraffin sections 5 micron thick were prepared and stained with hematoxylin and eosin stain (Survarna et al. 2013) and examined microscopically.

RESULTS

I-Parasitological Examination:

First stage

Out of total 320 fishs (160/species) were examined for intestinal parasites from 8 fish farms in Sharkia Governorate, 116 fish were positive for intestinal parasites with prevalence (36.25%). Prevalence of infestation in 8 farms in each species were illustrated in figure (1) Examination of 50 dissected fish were resulted in presence of some intestinal parasites in both fish species as follow:

In *Oreochromis niloticus*:

Out of 160 *Oreochromis niloticus* fish examined for helminth parasites (intestinal parasites) from 8 farms. 50 *Oreochromis niloticus* were infected by intestinal parasites with prevalence rate 31.25% from examined fish. Infestation with cestode (*Proteocephalus* sp larvae) in some farms (Figure A) and nematode (*Contraceacum* sp. Larvae) (Figure B) were demonstrated.

In *Clarias gariepinus*:

Out of 160 *Clarias gariepinus* fish from each farm, examined for helminth parasites (intestinal parasites) from 8 farms. Sixty-six *Clarias gariepinus* were infected by intestinal parasites with prevalence rate 41.25%. Infection with trematode (*Orientocreadium* sp.) (Figure C), cestode (*Polygonchobothrium* sp) (Figure D) and nematode (*Procamallanus* sp.) (Figure E) were demonstrated. Detailed results of prevalence rate of single and mixed infection in both *Oreochromis niloticus* and *Clarias gariepinus* were illustrated in table (1)

Eggs count mean for Cestode, Nematode and trematode in case of *Clarias gariepinus* only, all from 116 examined infected fish were calculated and tabled (table2).

II-Haematological analysis:

Results for hematological parameters represented in graph [(1a&1b) and (2a &2b)] showed that both *oreochromis niloticus* and *Clarias gariepinus* fish infected with parasites (group 2) revealed a significant decrease in the RBCs count, Hb concentration and packed cell volume; on the other hand, there was a significant increase in the WBCs when compared with control group (Group 1). Infected fish treated with anti-parasitic drugs (Group 3 and 4) showed a significant increase in RBCs count, Hb concentration and PCV% when compared with infected non-treated group. In the present study, lymphocytosis, eosinophilia, basophilia, were recorded in parasitic infected fish when compared with control group. Infected fish treated with anti-parasitic drugs (Group3 and 4) showed a significant decrease in WBCs, lymphocyte and eosinophil when compared with infected non-treated group.

III-Serum biochemical analysis:

Regarding to the biochemical parameters our results in graphs (1c & 2c) revealed that fish infected with parasites (Group 2) revealed a significant decrease ($P < 0.05$) in serum total protein, albumin and superoxide dismutase (SOD) when compared with control group (Group1). Infected fish treated with anti-parasitic drugs (Group 3 and 4) showed a significant increase in serum total protein , albumin and (SOD) when compared with infected non-treated Groups on the other hand infected non-treated Groups showed a significant increase in malondialdehyde activity and lysozyme activity comparing with control group and Infected fish treated with anti-parasitic drugs (Group 3 and 4) showed a significant decrease in malondialdehyde and lysozyme activity when compared with infected non-treated groups. There were no preferences for albendazole over levamisole and vice versa, as both had nearly similar ameliorative effects on the infestation

IV-Histopathological examination:

Clinical signs and macroscopical findings revealed general signs on infected *Oreo-*

chromis niloticus and *Clarias gariepinus* fish represented in emaciation, paleness of most of internal organs, distended gall bladder with bile and enlargement of abdominal cavities related to organs affection according to site of infestation while our microscopical lesions were numerous and affected mostly liver, stomach and intestine in both fish

Infestation with trematoda (*Orientocreadium sp*) larvae was demonstrated near intestinal serosa (Fig.4a) and in liver within fin capsule embedded in the hepatic parenchyma (Fig.4b) caused some lesions in examined organs of *Clarias gariepinus* only represented in shortening of intestinal villi (Fig.4a) and hydropic degeneration of hepatocytes (Fig.4c) while stomach showed congestion of submucosal blood vessels with submucosal edema and leucocytic cells infiltrations.

Infestation with Cestode (*proteocephalus sp** and *polyonchobothrium sp***) larvae in both *Oreochromis niloticus*, *Clarias gariepinus* consequently affected (intestine & liver)* and (stomach)** caused common microscopical lesions as mucinous degeneration of intestinal enterocytes (massive metaplasia of enterocytes to goblet cells) (Fig.4d) and submucosal oedema mainly in *Clarias gariepinus* infected with *polyonchobothrium sp*. while stomach which was the main affected organ suffered some lesions as gastric mucosal atrophy (Fig.4e), hyalinization of muscularis mucosa with congestion of submucosal blood vessels and leucocytic infiltration of mainly eosinophiles. Few hepatic lesions were recorded and represented in mild congestion and mild hepatic vacuolation. *Oreochromis niloticus* infected with *proteocephalus sp*. revealed intestine with submucosal edema, leucocytic cells infiltration of mainly lymphocytes and macrophages while larvae could be seen inside hepatic parenchyma with granulomatous and fibroin's reaction (Fig.5a). Stomach showed submucosal fibrosis with mild diffuse congestion of submucosal blood vessels.

Infestation with Nematode (*contracaecum sp** and *polyonchobothrium sp***) larvae in both *Oreochromis niloticus*, *Clarias gariepi-*

nus consequently affected (intestine, stomach and liver)* and (stomach & intestine)** exhibited some lesions in the affected organs at the site infestation which were severe fibrosis of gastric muscularis mucosa with leucocytic cells infiltration of mainly eosinophils (Fig.5b). Gastric mucosal hypertrophy was also detected. Liver showed severe congestion of blood vessels with or without diffuse vacuolation of hepatocytes (Fig.5c). Remnant of larvae could be seen embedded in the hepatic tissue surrounded with fibrous inflammatory reaction (Fig.5d). Diffuse atrophy of hepatocytes could also be observed. Fusion of intestinal villi with submucosal cellular infiltration. *Clarias gariepinus* infected with *polyonchobothrium sp* revealed almost similar lesions observed in stomach and intestine infected with *contracaecum sp*. and represented in intestinal submucosal edema, cellular infiltration and fusion of intestinal villi in addition to desquamation of other. Noticeable non infected common lesion was seen in stomach of *Clarias gariepinus* infected with *procamallanus sp* and represented in gastric mucosal hyperplasia with hypertrophy of other mucosal parts

Experimental groups of both (*Oreochromis niloticus* *and *Clarias gariepinus***) showed some changes related to treated groups, similar lesions and infection in group 2 (infected group) as those demonstrated in dissected fish of the first stage and exhibited liver infected with *Orientocreadium sp*. with diffuse vacuolation of hepatocytes. Intestine infected with *proteocephalus sp*. with submucosal edema and partial destruction of lamina propria (Fig.6a). Stomach infected with *procamallanus sp*. with gastric mucosal marked epithelial hyperplasia and hypertrophy of other mucosal parts (Fig.6b). liver infected with *contracaecum sp*. With vacuolation of hepatocytes (Fig.6c). Liver of (gp.3) with diffuse disorganization of hepatic parenchyma and with periductal oedema (Fig.6d). Liver of (gp.4) with mild vacuolation of hepatocytes and normal architecture. Intestine of (gp.4) with apparently normal mucosa and submucosa. Some cases revealed liver with mild hyperplasia of hepatopancreatic epithelium. mild and moder-

ate lesions were noticed markedly in treated groups compared with infected one.

DISCUSSION

In the present study multi-infestation in the same fish was recorded, *Proteocephalus sp.* was found in *Oreochromis niloticus* with prevalence rate 20%. Differ than that detected by **Onoja-Abutu, et al. (2021)** who reported prevalence rate 6.2%. and with almost near to that percent detected by **Outa et al. (2021)** who found *Proteocephalus sp.* in *Oreochromis niloticus* with prevalence rate 27.3%.

In the present study, one nematode *Contracecum sp.* Larvae in *Oreochromis niloticus* with prevalence rate 56%. The genera of nematode identified in the body of *Oreochromis niloticus* were *Contracecum* the most prevalent (54.4%), **Abiyu, et al. (2020)**. The prevalence of *Contracecum* of Nile Tilapia (*Oreochromis niloticus*) in Selected Fish farms, in Amhara Regional State was 58.8%, **Adugna, (2020)** near to our detected prevalence rate.

In the present study, *Orientocreadium sp.* found infected in *Clarias gariepinus* with prevalence rate 27.3%. The infestation by *Orientocreadium batrachoides* showed high prevalence in *Clarias gariepinus* (63.15%), **Taha, (2018)**. Total of 144 *Clarias gariepinus* samples were collected from fish markets at Qena province for parasitological examinations, they infected by *Orientocreadium batrachoides* with prevalence rate (24.3%), **El-Seify, et al. (2017)**.

In the present study, *Polyonchobothrium sp.* found infected in *Clarias gariepinus* with prevalence rate (13.6%). Total of 144 *Clarias gariepinus* were collected from fish markets at Qena Governorate *Polyonchobothrium clarias* with prevalence rate (5.6%), **El-Seify, et al. (2017)**. The helminth parasites investigation from Qalubia governorate in *Clarias gariepinus*, *Polyonchobothrium clarias* was found infected with prevalence rate (6.67%), **Ali, et al. (2020)**.

In the present study, *Procamallanus sp.* found infected in *Clarias gariepinus* with prevalence rate (31.8%). The investigation of hel-

minth parasites from Qalubia governorate in *Clarias gariepinus*, *Procamallanus laeviconchus* was found infected with prevalence rate (33.3%), **Ali, et al. (2020)**. Total of 144 *Clarias gariepinus* were collected from fish markets at Qena Governorate *Procamallanus laeviconchus* with prevalence rate (5.6%), **El-Seify, et al. (2017)**. High prevalence of some parasites could be attributed to presence of mixed infestation that registered in both species

Hematological parameters:

Results of RBCS count, Hb concentration and PCV% showed significant decrease these results agree with **Martins et al. (2004)** **Kundu et al. (2016)** these changes may be due to the fact that parasitic infestation leads to anemia, and parasites itself act as a stress factor, more over changes in PCV attributed to release of catecholamine, which can mobilize RBCs from spleen or cause fluid shift into the intracellular compartment leading to red blood cell swelling, Similar results were recorded by **Sabri et al. (2009)** & **Nabuchi et al (2015)**. More over **Lofty et al (2003)** attributed the reduction of RBC, PCV and Hb to chronic liver inflammation which causes depression of erythropoiesis leading to severe anemia. In the present study significant increase in WBC count was found in parasitized fish that due to the fact that WBCs play a major role during infestation by stimulating the immune system and haemopoietic tissues to produce antibodies and chemical substances which have defense mechanism against infection (**Khurshid & Ahmad, 2012**).

In our study a significant increase in heterophils and eosinophil these results agree with **Wakenell, (2010)** mentioned that heterophils infiltration increase immediately after infestation as a first defense mechanism followed by increase in eosinophil concentration as a response to parasitic infestation, more over **Rosenberg et al. (2007)** reported that During parasitic infestation, the numbers of peripheral blood eosinophils are highly increased under the influence of T helper -2 cell-derived IL-5 and IL-3, and eosinophils are recruited from the circulation into inflamed or damaged tissues by the eosinophil selective chemokine. Treatment of infected fish with anti-parasitic

drugs resulted in improvement in hematological parameters, our results in accordance with **Speare et al. (1999)** who reported that albendazole was effective against *loma salmonae*, also **Alves et al. (2019)** reported that levamisole was 100% effective as therapeutic baths in parasitic infected fish.

Serum biochemical analysis:

In this study, a decrease in the serum protein and albumin values of the parasitized fish, these findings agree with **Çelik & Aydın, (2006)** whom reported a decrease in total protein value as a result of long fasting and various distress factors. Additionally, **Bhaktavathsalam and Srinivasa, (1984)** stated that parasitic infestation causes proteolysis of serum protein, which results in the liberation of free amino acids and necrosis of hepatocytes, additionally this decline may be attributed to the increased level of transaminase activity, indicating the rapid utilization of reserve foods like protein and carbohydrate under stress conditions. Another point of view **Eissa et al., (2010)** stated that the decrease in total protein can be attributed to the consumption of the nutrient materials by parasites and inhibition of protein and nutrient absorption in the nutrient materials

Antioxidants are very useful in the evaluation of health status and have been widely used as biomarkers of oxidative stress in fish that were exposed to xenobiotics in experimental conditions or inhabited polluted environment (**Rudneva et al. 2012; Kaptaher et al. 2014**).

Parasite invasion is act as stress factor on organism and may stimulate reactive oxygen species (ROS) production. In the present study the parasitized fish showed a significant increase in malondialdehyde activity and a significant decrease in the level of SOD, our results in agree with [**Mikraykov & Silkina, (2006)** and **Skuratovskaya & Zav'yalov, (2006)**] whom reported a decrease in total antioxidant activity and increase of lipid peroxidation. Our results disagree with **Skuratovskaya and Zav'yalov, (2018)** who reported an elevation in the level of antioxidant enzyme in parasitized black Sea whiting Mer-

langius, that explained by that the response of antioxidant system in parasitized fish is not uniform and it depends on parasite species, tested tissues and specificity of enzyme (**Hursky and Pietrock, 2015**).

Fish lysozyme is an exceptionally widespread defense molecule of the innate immune system, which is important for protection against fish pathogen (**Saurabh and Sahoo, 2008**). In the present study the parasitized non treated fish evoked a significant increase in lysozyme activity, our results agree with **Rashed et al. (2021)** reported that in parasitic infestation the level of lysozyme increase to overcome the stress conditions associated with infestation, our results disagree with **Khalil et al. (2018)** and **Karagouni et al. (2005)** who reported a decline in lysozyme activity in parasitized fish.

Histopathological examination with macroscopical alterations in examined organs of *Oreochromis niloticus* were in partial accordance with the observations of **Mathenge, (2010)** and with far similar to those in examined organs of *Clarias gariepinus* detected by **Radwan et al. (2021)**. Tissue damage in the infected organs could be attributed to the direct effect of localized parasites causing spontaneous irritation in the site of infestation as in digenetic trematodes (**Noga, 2010**).

Presence of larvae as a foreign antigen inside the host tissues as in *Orientocreadium* in the intestinal tissue and hepatic parenchyma provoke its immune system to trigger the inflammatory elements as a defense against, hence inflammatory reaction occur including vascular congestion, tissue edema and cellular infiltration. Intestinal parasites provoke structural modification of host tissue and induce alterations in normal intestinal physiology as impairment in process of digestion and absorption of food, water and electrolytes (**Hoste, 2001**) which explained the mucinous degeneration and excessive mucous secretion into lumen.

Our observed lesions in infected liver, intestine and stomach in both digenetic trematodes

(*Orientocreadim* sp) and cestode (*Polyonchobotrium* sp) were in partial accordance with those obtained by (Walaa et al. 2012) and with (Eissa et al. 2012) who observed similar lesions in liver, intestine and stomach of *Clarias gariepinus* infected with cestode (*Polyonchobotrium* sp).

The alterations observed in histopathology of the vital organs of fish due to experimental *Procamallanus* infestation appear to be induced by excretory and secretory metabolites (exotoxins/endotoxins) produced by the parasites harbouring the intestine. (Shashi Ruhela et al. 2012) while lesions observed in (*Clarias* spp. and *Heterotis niloticus*) infected with *proteocephalus* sp by Wabuke -Bunoti, (1980) were in trend with our results, the author mentioned that these worms pose a potential risk to these native fish species and reported some tissue response (inflammation) around bothria of *P. clarias* attached to gut mucosa in infected Lake Victoria *C. gariepinus*. In the same fish, however, bothridial penetration into the gall bladder mucosa causes pronounced nodules. Such nodules contained granulomatous and fibrous tissue. Reduction of lesions intensity in farms applied prophylactic regimens could be attributed to the drug action on helminth larvae in reduction of heavy infestation in both larval number and its migration inside fish organs.

CONCLUSION:

Parasitic diversity of intestinal infestation in both *Clarias gariepinus* and *Oreochromis niloticus* with considerable prevalence rate in summer season were recorded. The effectiveness of applying both commonly used anti parasitic drugs (Albendazole and levamisole) in control of intestinal helminth in fish.

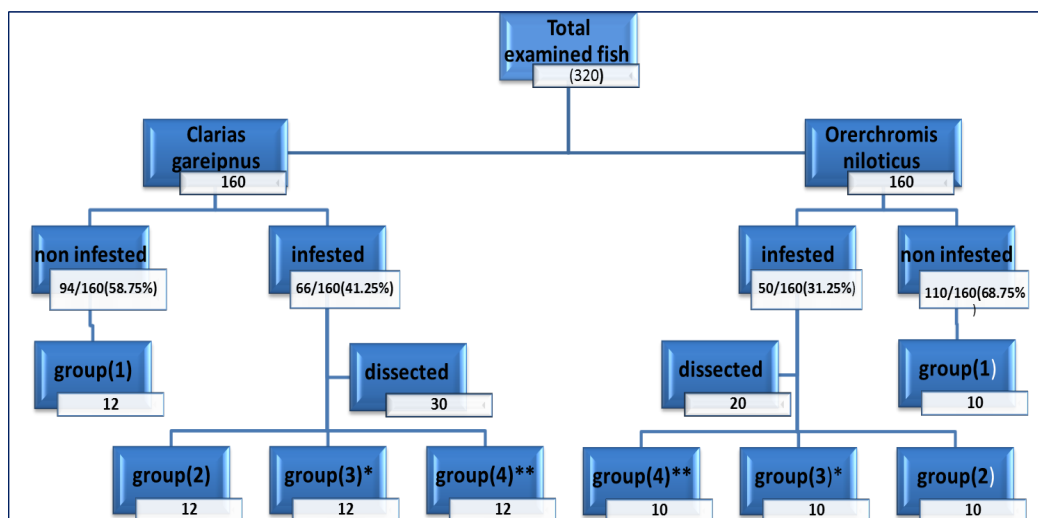


Figure (1) Diagram for protocol design of the whole study.

(*) Albendazole treated group

(**) Levamisole treated group

Table 1. Prevalence rate of single and mixed infestation in both *Oreochromis niloticus* and *Clarias gariepinus*:

<i>Oreochromis niloticus</i>		<i>Clarias gariepinus</i>								
Single Infection		Mixed Infection		Single Infection		Mixed Infection				
<i>Proteocephalus</i>	<i>Contra-ceacum</i>	<i>Proteo.</i> +	<i>Contra.</i>	<i>Ori-entocreadium</i>	<i>Polyon-chobothrum</i>	<i>Pro-camalanus</i>	<i>Orien.</i> +	<i>Orien.</i> +	<i>Poly.</i> +	<i>Orien.</i> +
10 (20%)	28 (56%)	36 (72%)		18 (27.3%)	9 (13.6%)	21 (31.8%)	26 (39.3%)	14 (21.2%)	4 (6%)	17 (25.7%)

Table 2. Egg count mean (first stage) from 116 examined infected fishes in 8 farms

parasite	Fish <i>Clarias gariepinu</i>	<i>Oreochromis niloticus</i>
Cestode	48	29
Nematode	86	43
Trematode	22	0

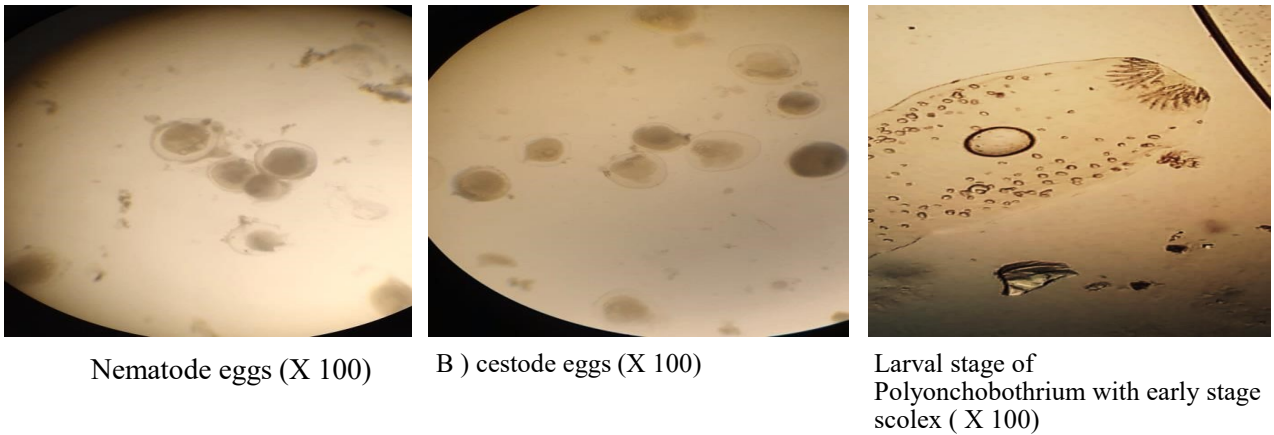


Figure 2. photomicrographs of some examined fecal samples for parasitological examination eggs and larvae



A) *Proteocephalus* sp
a-*Proteocephalus* sp.:
Phylum: Platyhelminthes
Class: Cestoda
Order: Proteocephalidea
Family: Proteocephalidae
Genus: *Proteocephalus* sp. Larvae
Site of infestation: Intestine



B) *Contraceacum* sp. Larvae
b- *Contraceacum* sp. Larvae:
Phylum: Nematoda
Class: Chromadorea
Order: Rhabditida
Family: Anisakidae
Genus: *Contraceacum* sp.
Site of infestation: Intestine & Body cavity



(C) *Orientocreadium* sp
Phylum: Platyhelminthes
Class: Trematoda
Order: Digenea
Family: Orientocreadium
Genus: *Orientocreadium* sp
Site of infestation: Intestine



(D) *Polyonchobothrium* sp.
Phylum: Platyhelminthes
Class: Cestoda
Order: Pseudophyllidea
Family: Bothriocephalidae
Genus: *Polyonchobothrium* sp
Site of infestation: Stomach



E) *Procammallanus* sp.
Phylum: Platyhelminthes
Class: Nematoda
Order: Spiruridea
Family: Camallanidae
Genus: *Procammallanus* sp
Site of infestation: : Intestine

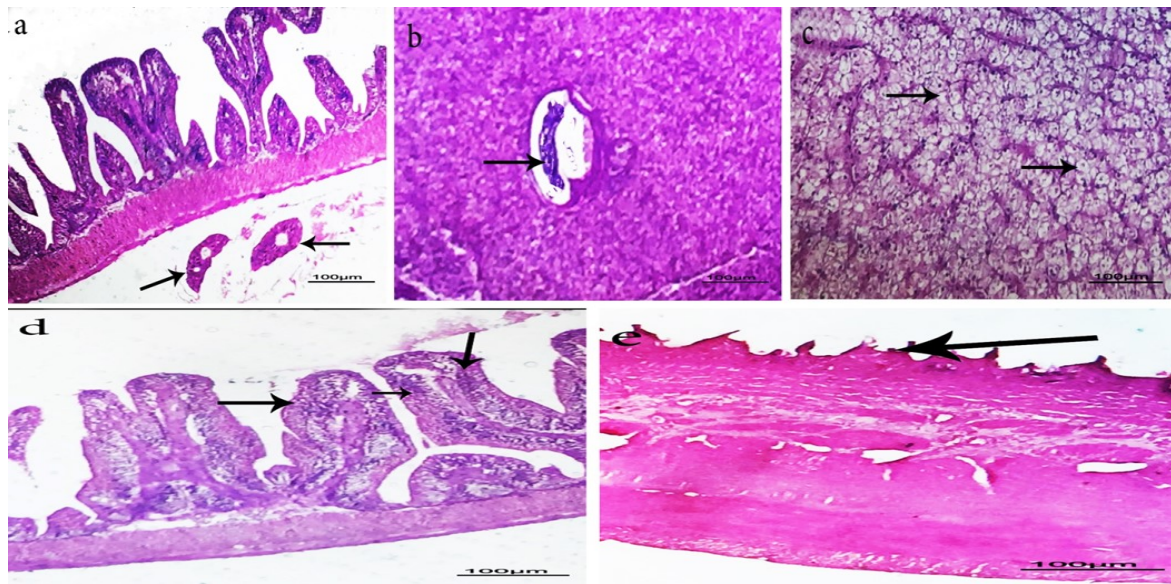


Figure (4) Photomicrograph of H&E stained section of infected *Clarias gariepinus and *Oreochromis niloticus* ** a)* intestine infected with *Orientocreadium sp.* showing larvae near intestinal serosa (arrows) with shortening of intestinal villi (arrowhead). b)* liver infected with *Orientocreadium sp.* showing larvae within fin capsule embedded in the hepatic parenchyma (arrow) c)* liver infected with *Orientocreadium sp.* Showing diffuse vacuolation of hepatocytes (arrow) d)* intestine infected with *polyonchobothrium sp.* showing mucinous degeneration of intestinal enterocytes (arrows) e)* stomach infected with *polyonchobothrium sp.* with gastric mucosal atrophy (arrow) (scale bar =100 μ m)**

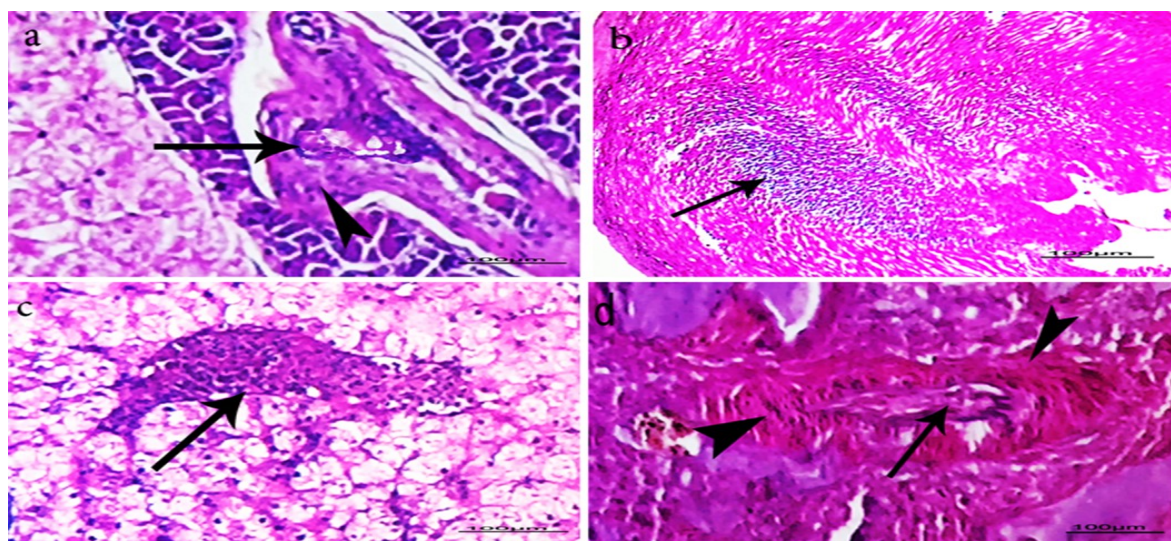


Figure (5) Photomicrograph of H&E stained section of infected *Oreochromis niloticus* *and *Clarias gariepinus* a) liv with *proteocephalus sp* showing granulomatous reaction (arrowhead) surrounded the larvae infected hepatic parenchyma (arrow) with granulomatous and fibrous reaction (arrow head) b) * stomach infected with *contraeaecum sp.* showing severe fibrosis of galaris mucosa (arrow) c)* liver infected with *contraeaecum sp.* showing severe congestion of blood vessels (arrow) with diffuse vac hepatocytes d)* liver infected with *contraeaecum sp.* Showing presence of remnant of larvae (arrow) embedded in hepatic parenclated with fibrous inflammatory reaction (arrows head) (scale bar =100 μ m)**

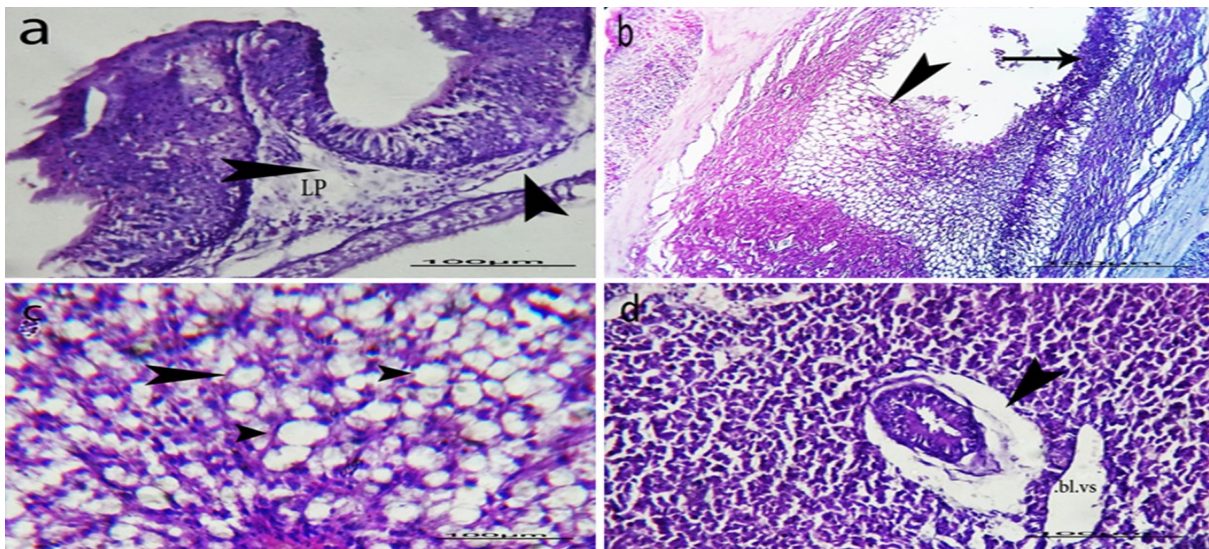
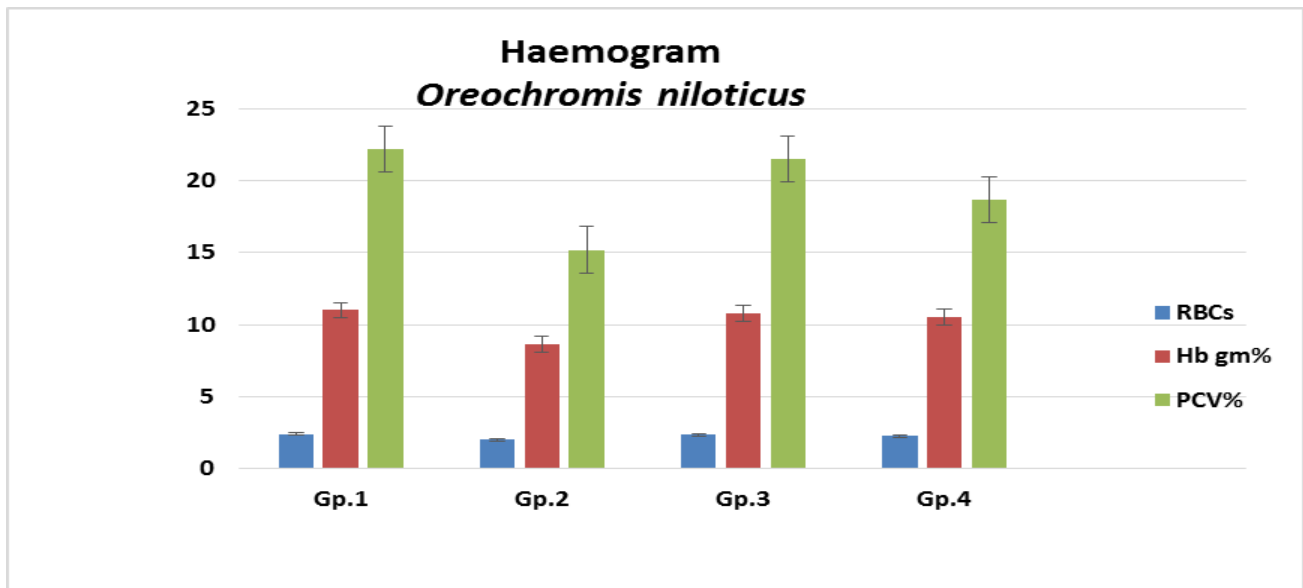
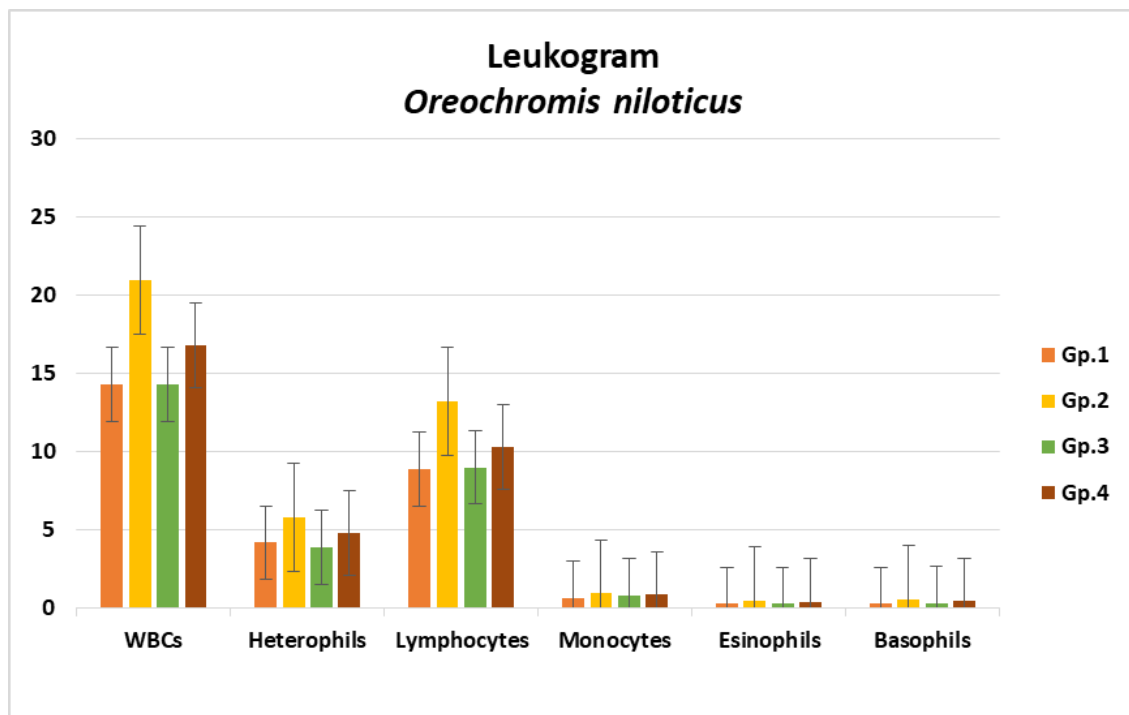


Figure (6) Photomicrograph of H&E stained section of experimental groups of both *Oreochromis niloticus* *and *Clarias gariepinus*** .a)* intestine of (gp.2) infected with *proteocephalus sp* .showing submucosal edema (arrowshead) with partial destruction of lamina propria . b) ** stomach of (gp.2) infected with *procamallanus sp* .showing variable degrees of epithelial hyperplasia (arrowhead) with hyperatrophy of other mucosal parts (arrow) .c)* liver of (gp.2) infected with *contraecum sp* .showing diffuse vacuolation of hepatocytes (arrows head) . d)** liver of (gp.3) showing mild periductal edema (arrow head) . (scale bar =100 μm)

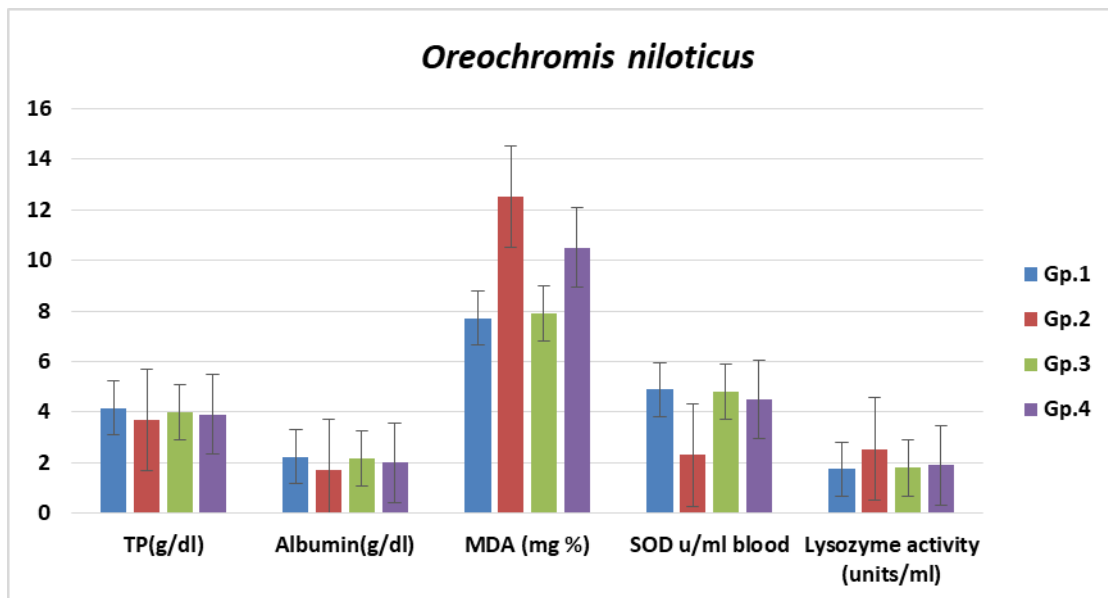


Graph (1a) Mean values of hemogram parameters in *Oreochromis niloticus* fish after treatment (first stage):

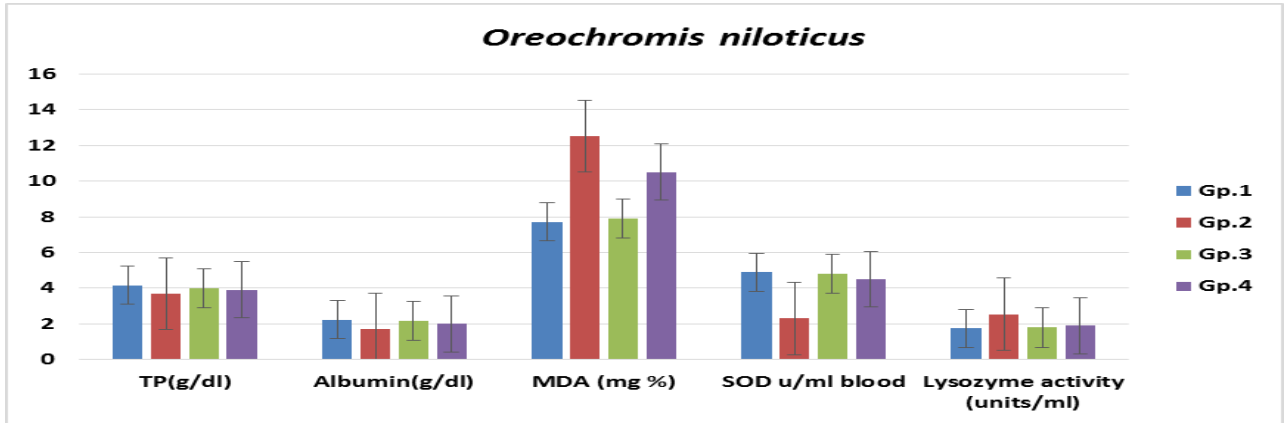
significantly different at $P < 0.05$ with error bars



Graph (1b): Mean values of leukogram parameters in *Oreochromis niloticus* fish after treatment (first stage): significantly different at $P < 0.05$ with error bars

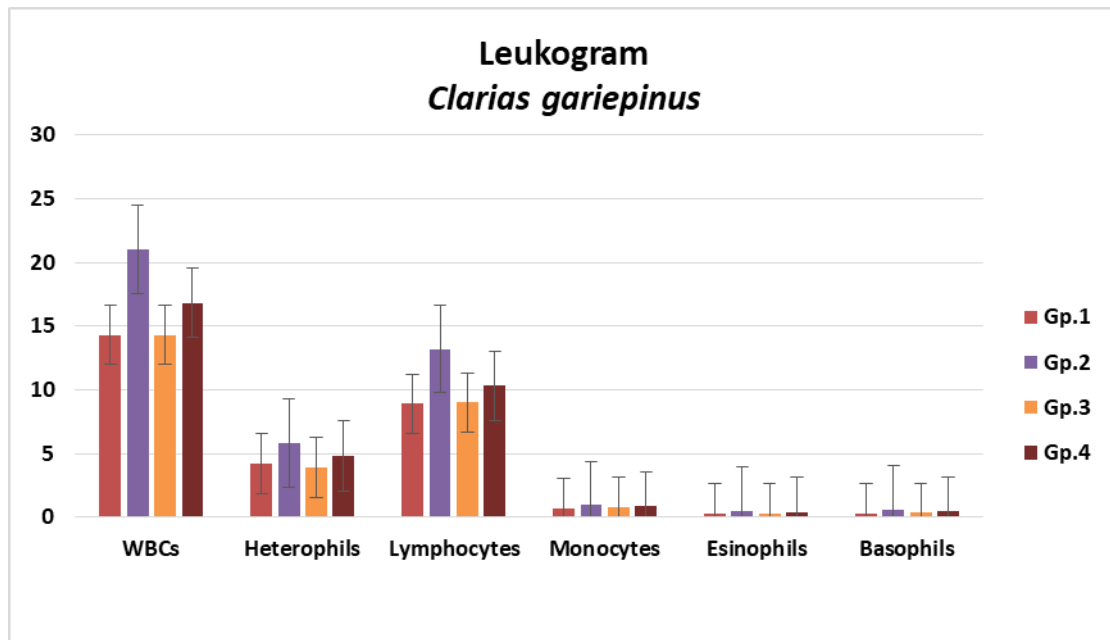


Graph (1c) : Mean values of biochemical parameters in *Oreochromis niloticus* fish after treatment (first stage): significantly different at $P < 0.05$ with error bars

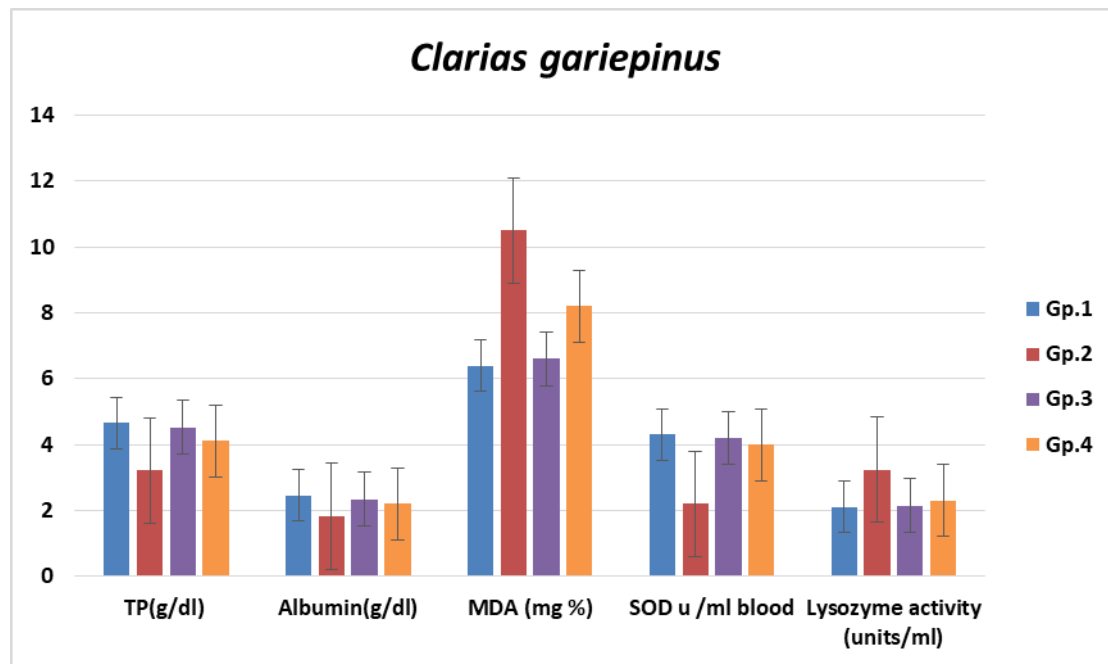


significantly different at P < 0.05 with error bars

Graph (2a) : Mean values of hemogram parameters in *Clarias gariepinus* fish after treatment (second stage)



Graph (2b): Mean values of leukogram parameters in *clarias gariepinus* fish after treatment (second stage):



Graph (2c) : Mean values of biochemical parameters in *Clarias gariepinus* fish after treatment (second stage) Significantly different at $P < 0.05$ with error bars

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