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Effects of parasites infection on fishes collected from Daim Mayo lagoon at the Sudanese coastal waters

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Abstract
This study aimed to investigate the effects of parasites infection on tissues and biometrics of fishes collected from the Daim Mayo lagoon. The study was conducted in the period from April 2014 to May 2015. The results obtained indicated significant differences in biometric parameters between healthy and infected fishes ($p< 0.05$). Values of liver somato index, gonado somato index and condition factor were higher in healthy fishes. Hypertrophy and Hyperplasia of gill lamellae were detected in the gill filaments of fishes Mugil cephalus and Rastrelliger kanagurta due to infection by Lernanthropus sp., Aella sp. and Gnathia sp. parasites. The presence of necrosis and edema were detected in the mouth roof of Mullets M. cephalus due to infection by Cymothus sp. Damage to the intestinal villi, lymphocytes infiltration, edema, focal necrosis. Haemorrhage and separation of submucosa layer from muscular layer were observed in the intestine of fishes Chanos chanos and M. cephalus that were infected by nematode Procamallnus sp. On the other hand, parasitize intestine of M. cephalus fishes with acanthocephalan Sclerocollum sp. showed lymphocytes infiltration and congestion in arteries, damage in intestinal villa and thickness in the muscular layer. The liver of Caranoide flavogattus and M. cephalus fishes showed histopatholgical alteration in tissues which include focal necrosis in the liver parenchyma, haemorrhages, melanomacrophages aggregation and atrophy of liver sinusoid.

Keywords: Parasites, fishes, Sudanese coast

1. Introduction
The fisheries sector has the potential to contribute to food security as well as the economy of the Red Sea State, nevertheless the marine fishery is still considered under developed. However, the marine fisheries potential was estimated as 10,000tons/year, while the production reported was 5000tons/year [1] Fisheries contribution in food security for the Red Sea State indicated that 93.8% of the studied population was used to have fish in their diet compared to 66.4% of those who used to have animal meat. However, a value of 9.6 Kg was calculated as the average fish consumption per capita for the Red Sea State [2].
Parasites are an important group of pathogens, which occurs at various stages of development in fish. The parasites attack various tissues and organs of fish, e.g. skin, gills, eye, kidney, liver, intestine, spleen, heart and brain. Parasitic infection tends to decrease the growth rate resulting in stunning of fishes. Parasites are metabolically dependent on their host mainly for their nutritional requirements [3].
The complex relationship between hosts and parasites depends on a number of factors. In principle, the parasite seeks to establish itself in the host while the host resists the invasion through its defense mechanism [4].
Studies on the cellular response to the parasites in the last two decades had been limited to the description of the histopathological lesions produced by the parasites, and changes in leucocytes in blood or infected sites. In many cases, the granulomas, in which the parasite and their products are encapsulated, were formed as an inflammatory response and it is one of the recognized mechanisms of immune evasion [5, 6].
Nematodes intestinal parasites can cause a wide range of damages, including tissue degradation, hemorrhage, inflammation, granulomas and mesenteric and visceral adhesions. It was also suggested to cause primary anemia by feeding on blood. In mass infections, especially in small fishes, some nematodes cause intestinal blockage and may reduce growth rates [7].

The effect of acanthocephalan infection includes mucus cell hyperplasia, which commonly occurs in acanthocephalan infections. The covering resulting from the copious secretion of mucus and the presumed presence of antibodies within may probably reduce the number of parasites that succeed in establishing [8].

[9] stated that in low to moderate infestation by acanthocephalan, pathological effects are localized around the proboscis site of the adult worm. The extent of damage is proportional to the depth of penetration of the proboscis. It is negligible when parasites are attached to the epithelial mucosa, and becomes extreme, with extensive granuloma and subsequent fibrosis, when the worm's proboscis is anchored in the muscle layer or entirely perforates the intestinal wall (*Pomphorhynchus sp*.).

The acanthocephalan *Sclerocollum sp*. damage the architecture of intestinal tissues beside hemorrhage, hyperplasia in mucosa and submucosa; melanomacrophage aggregation and necrosis of mucosa and a sub-mucosal layer of *S. rivulatus* [10].

The copepod parasites cause changes on the gill filaments through feeding. The gill lamellae, gill arches and gill rakers were badly damaged [11].

[12] stated that copepod parasites attach to the host using various appendages modified for grasping; this attachment can lead to secondary infection by a pathogenic organism such as bacteria, fungi, and viruses and cause mass mortality. The high intensity of infection of these copepods may lead to serious damage of the gills and therefore show a pronounced impact on the histology and lead to mortality [13].

The copepod Lernanthropus *sp*. can often cause histopathological effects like desquamation, erosion, and necrosis of the host's gill filaments [14]. Heavy infestation may lead to asphyxiation, anemia and secondary bacterial infections [15].

The attachment and feeding activities of *Aella sp*. on black sea bream resulted in hyperplasia of the gill lamellae [16].

Adult females of Cymothoids are either attached to the skin, gills, buccal cavity or burrow into the fish and develop in a pouch-like most isopods, Cymothoids are considered to feed principally on host blood, but they may consume the mucus, epithelium and subcutaneous tissues of their hosts [17].

This study was proposed to investigate the impact of fish parasites on fish health by means of biometric factors and tissue histopathogical structures of fish that collected from the Daim Mayo lagoon at Port Sudan harbor along the Sudanese Red Sea Coast.

2. Materials and Methods

2.1 The study area

This study was carried out during the period from April 2014 to May 2015 in the Daim Mayo lagoon. It is semi-enclosed, elongated lagoon, extending from the sea inland to about 5.5 km long and approximately 1.0km wide at the main basin and terminates into a shallow lagoon (≤500m wide).

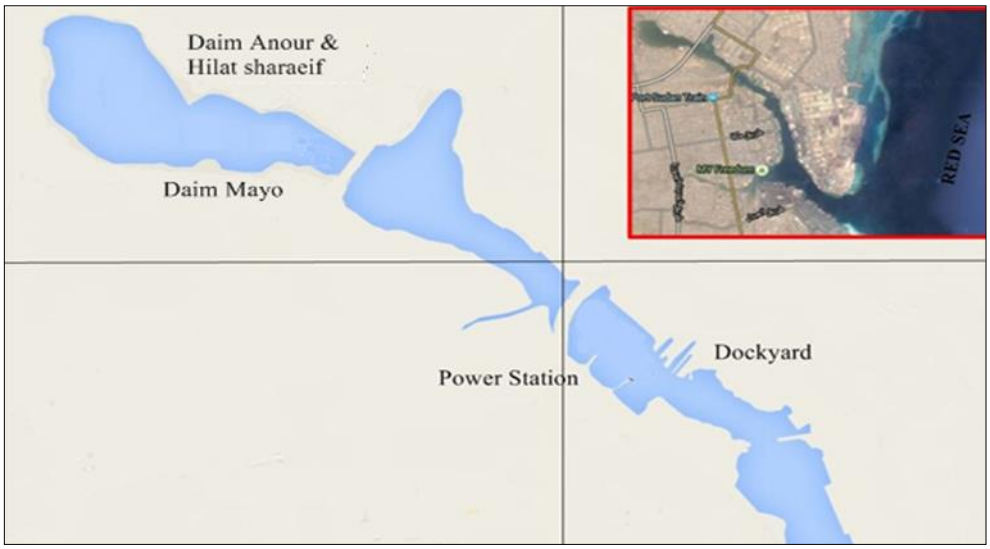

Fig 1: Study area. Modified from www.flashearth.com (2015).

2.2 Fish sampling

164 Fish samples were collected randomly via gill net with 6 cm mesh size and of 4 to 5 m lengths twice a month, the collection was carried out during early morning, fish were identified according to [18, 19].

2.3 Biometric parameters

The length was measured using a metric ruler to the nearest centimeter, and weight was measured using digital balance to the nearest grams according to [20].

2.3.1 Calculation of hepatosomatic index measurement

The liver somato Index was calculated based on the method described by [21].

$$L.S.I = \frac{Liver\ weight}{Body\ weight} \times 100$$

2.3.2 Calculation of gonadosomatic index

This was calculated as a percentage of the gonad weight to body weight according to [22].

$$GSI = \frac{Gonad\ Weight}{Total\ weight\ of\ the\ Fish} \times 100$$

2.3.3 Calculation of condition factor

The method of calculating the condition factor 'K' is given according to [23].

$$GSI = \frac{Body\ Weight}{Total\ length^3} \times 100$$

2.4 Histology Sample Processing
Tissue samples were cut from the same morphological section of each organ- to allow comparative analysis-. Preserved in 10% of formalin, after 24 hours of fixation, tissues were prepared for serial sectioning. The specimens were stained by Haematoxylin and leosin [24].

3. Results and Discussion
3.1 Biometrics of healthy and infected fishes
Biometric parameters results of healthy and infected fishes were presented in Table. 1, however the biometric values for all species studied showed significant differences ($p< 0.05$) between healthy and infected fishes.

Biometric values of liver somato index, gonado somato Index and condition factor of M. cephalus and R. kanagurta infected in gills with Lernanthropus sp, Aella sp. and Gnathia sp. ecto-parasites were significantly different ($p< 0.05$) compared to healthy ones. Similarly, the differences in biometric values between M.cephalus infected in mouth roof with Cymothus sp. parasites and healthy ones were significant ($p< 0.05$). Also, there were significant differences ($p< 0.05$) in biometric values of C. chanos and M. cephalus infected with Procamallanus sp. and Sclerocollum sp. parasites compared to healthy ones (Table. 1).

[25] Mentioned that heavy parasites infection can cause haemorrhage, but it may not necessarily always disrupt fish growth. Parasites effect on morphometric parameters became markedly unclear due to the complex of the marine environment and the effects of other factors like salinity, temperature, spawning season [26].

Table 1: Mean biometric values of healthy and infected fish species:

Species / Variable	*M. Cephalus*		*R. kanagurta*		*C. chanos*	
	Healthy	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**
L. S. I (%)	1.98±0.29^a	1.79±0.05^b	1.13±0.35^a	0.87±0.17^b	1.09±1.5 ^{a}	0.99±0.0 ^{b}
G. S. I (%)	0.69±0.18^a	0.37±0.22^b	1.5±0.15 ^{a}	1.06±0.86 ^{b}	0.8±0.27 ^{a}	0.5±0.0 ^{b}
C.F (%)	1.11±0.29 ^{a}	0. 9±0.02 ^{b}	1.60±0.20 ^{a}	1.40±0.03 ^{b}	4.93±0.02 ^{a}	1.37±0.0 ^{b}

Means ± (SE) within same column followed by different superscript small letters are significantly different at ($P< 0.05$) based on t-test. Abbreviations Used: L. S. I=Liver somato Index, G. S. I= Gonado somato Index, C. F=Condition factor

3.2 Effects of parasites infection on fish tissues
Histopathological alterations like hypertrophy and hyperplasia of gill lamellae (Fig.2, Fig.3) were detected in the gill filaments of infected M. *cephalus*, this was also reported by [11] and [12]. Lernanthropus sp. can often cause pathological effects like desquamation, erosion, and necrosis of the hosted gill filaments [14]. Atrophy of gill lamellae and lamellae fusion of R. kanagurta were due to infection by Ganthia sp. (Fig.4). Also, Gnathiid parasites caused changes on the gill filaments through feeding. Both the gill lamella and the gill arch and gill rakers were badly damaged as recorded by [11].

In this study presence of necrosis and edema were detected in the mouth roof of M. cephalus fish infected by Cymothus sp. (Fig.5), this in agree with [17] who mentioned that Cymothoids are considered to feed principally on host blood, but they may consume the mucus, epithelium and subcutaneous tissues of their hosts leading to necrosis.

Damage of the intestinal villi (Fig.6) was observed in the intestine of M. cephalus due to Procamallnus sp. infection, similarly this was described by [7]. However, lymphocytes infiltration was also observed (Fig.7), which in agree with [27] and [28]. Procamallanus sp. also caused edema, focal necrosis, haemorrhage, however [29] mentioned that the intestinal wall grabbed with buccal capsules of procamallanus sp. may lead to haemorrhage and more over separation of submucosa layer from a muscular layer which was observed in the intestine of infected fishes (Fig.8). Hyperplasia of the mucosa layer might be due to parasites attachment which leads to the formation of space between the muscular layer and submucosa layer [30]. Procamallanus sp. in the intestine of C. chanos showed haemorrhage and separation of the submucosa layer from the muscular layer (Fig.8), the same results were presented by [27, 28]. All cross-sections of villi revealed hemorrhages because the intestinal walls were grabbed by buccal capsules of Procamallanus sp. while feeding on blood.

The pathological effect of Sclerocollum sp. in the intestine of M. *cephalus* include lymphocytes infiltration and congestion in arteries (Fig. 9), damage in intestinal villa and thickness of the muscular layer, Fig. (10). The same effect was reported by [10]. In this study, although intensive intestine parasites were detected in fish intestine, never the less no signs of illness were observed, however [31], mentioned that in heavy parasite infections, the intestine appeared to be packed with parasites, almost blocking the intestinal lumen, which will adversely affect the movement of digested materials within the intestine and the absorption of nutrients.

Histological changes reported in the livers of most fish species collected from the study site were attributed to adverse environmental impacts resulting from wastewater discharges from the desalination plant, or from human activities rather than parasite infection. [32] stated that the effect of polluted water on fish tissues is clearer in liver tissue because the liver carry the task of detoxification and biotransformation and excretion of xenobiotics.

The presence of necrosis in liver parenchyma of C. flavogattus fish (Fig.11) and haemorrhages (Fig.12) in the liver of M. cephalus were recorded, these were also observed in the siluriform Corydoras paleatus contaminated by organophosphate pesticides [33] and [34]. Melanomacrophages aggregation in liver sinusoid of M. cephalus were present Fig (13), the function of the melanomacrophages and hemosiderin in the liver of fishes remains uncertain, but some studies have suggested that it is related to destruction, detoxification or recycling of endogenous and exogenous compounds [35]. An increase in the melanomacrophages aggregation number, size or hemosiderin content was reported in fish collected at contaminated sites when compared to those collected at reference sites [36, 37] and [38]. Atrophy of liver sinusoid Fig. (14) of C. flavogattus was also reported. [39] studied the effect of pollutants on the histological structure of the liver of the fish S. rivulatus collected from the Red Sea coast of Saudi Arabia he presented hepatocyte vacuolization and ballooning, cellular degeneration and coagulative necrosis, cellular infiltration, granuloma inflammation and bile duct

proliferation.

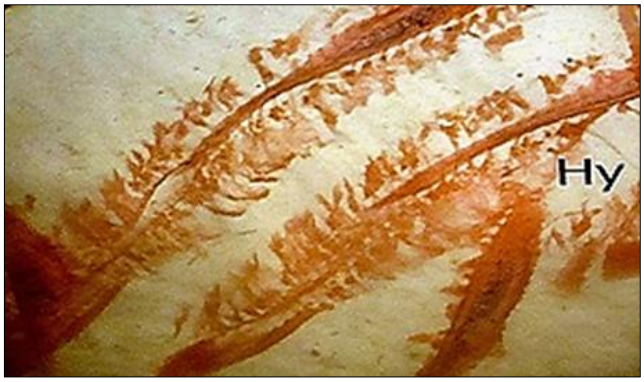

Fig 2: Hyperplasia of lamellae (HY) of *Mugil cephalus* infected by *Lernanthropuss sp* H&E. 10.x

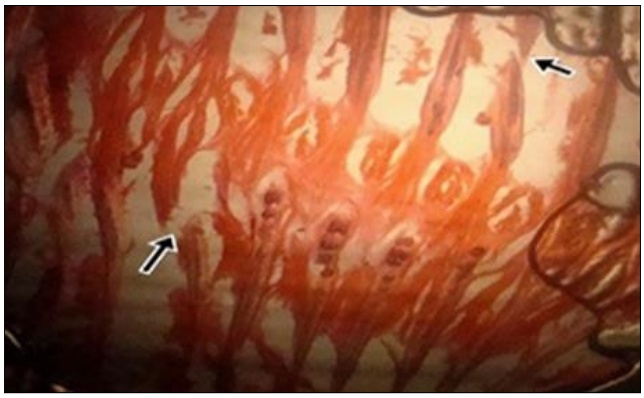

Fig 3: Damage in gill filaments (Arrow) of *Mugil cephalus* infected by *Ganthia sp.* H&E.10x

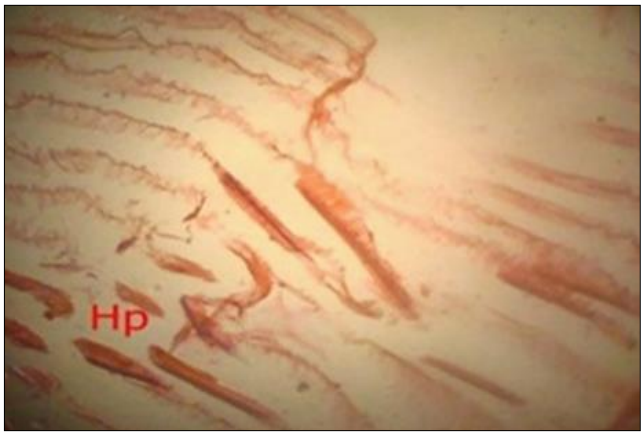

Fig 4: Atrophy of gill lamellae and lamellae fusion (Hp) of *Rastrelliger kanagurta* fish infected by *Ganthia sp*. H&E. 10x

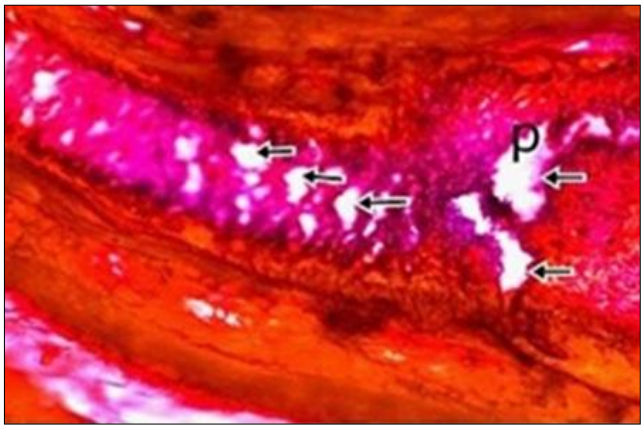

Fig 5: Necrosis and edema inside mouth roof of *Mugil cephalus* infected by *Cymothus sp*. H&E. 10x

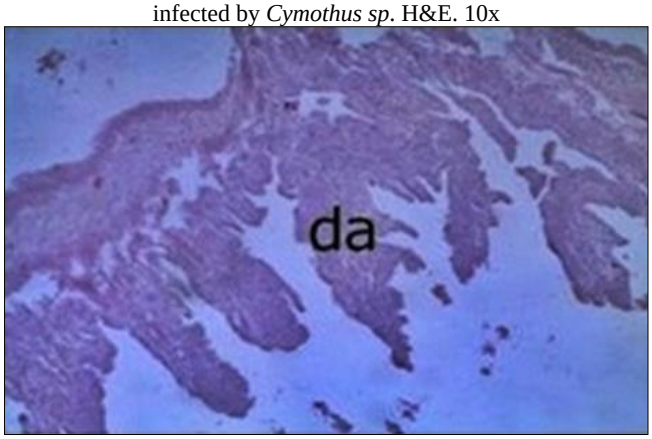

Fig 6: The damage of the intestinal villi (da) of *Mugil cephalus* infected by *Procamallnus sp*. H&E. 40x.

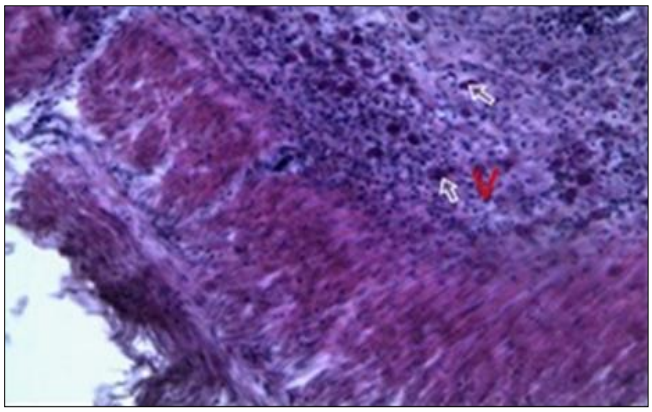

Fig 7: Lymphocytes infiltration (V) Inside submucosa of Mugil cephalus infected Procamallnus sp. H&E. 40x.

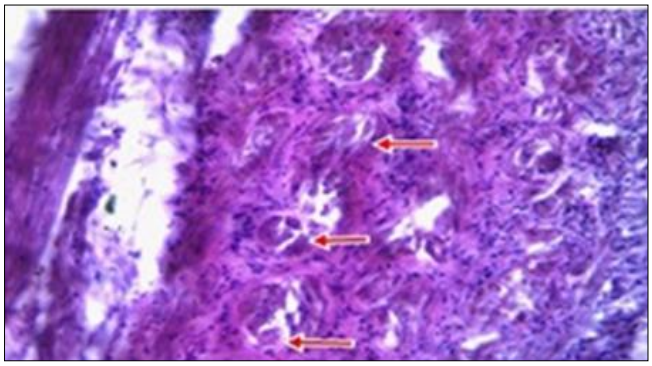

Fig 8: Sub mucosa layer showing haemorrhage and separation of from muscular layer of Chanos Chnos infected by Peocamallnus sp. H&E. 40x.

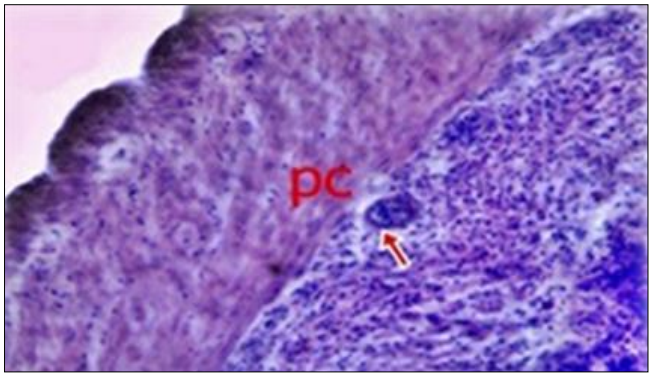

Fig 9: Submucosa layer reveal lymphocytes infiltration and congestion in arteries (Pc) of mugil cephalus due to sclerocollum sp. infection. H&E. 40.x

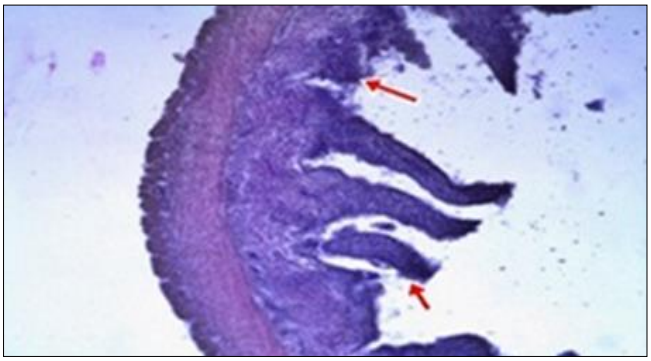

Fig 10: damage in intestinal villa (arrow) and thickness in muscular layer of *Mugil cephalus* infected by *Sclwrocollum sp*. due to infection. H&E.

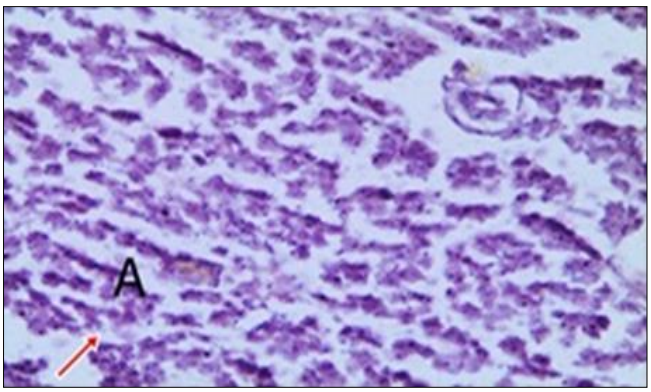

Fig 11: Liver of Paranoid flavogattus fish showing necrosis (arrow) in liver parenchyma. H&E. 40x.

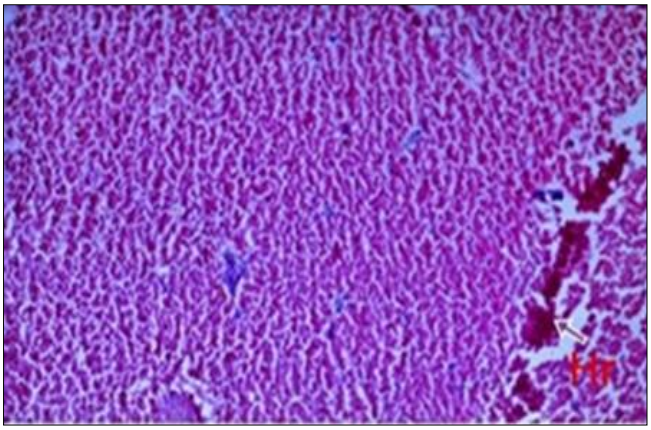

Fig 12: Hemorrhage in liver of *Mugil cephalus* (Hr.) H&E. 10x.

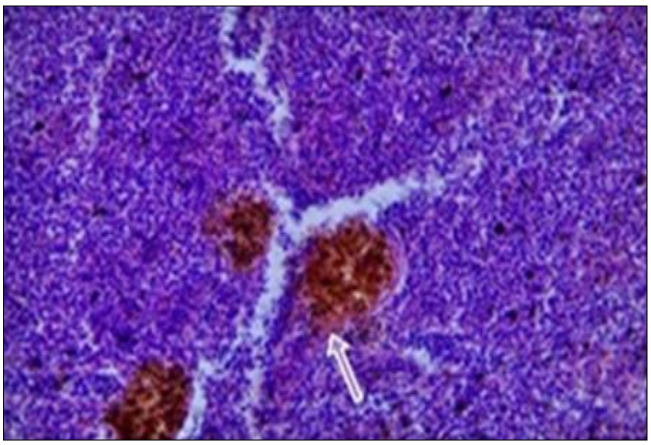

Fig 13: melanomacrophages aggregation in liver sinusoid (arrow) of Mugil cephalus fish infected. H&E. 10x.

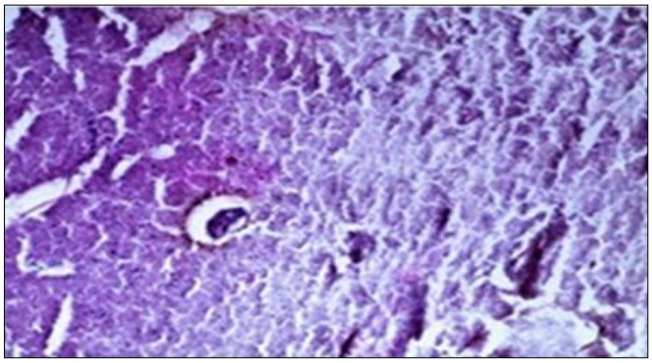

Fig 14: Atrophy of liver sinusoid of *Carangoides flavogattus* fish. H&E. 40x.

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