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BLOOD FLUKE PARASITIC DISEASE (SANGUINICOLOSIS) AFFECTING CLARIAS GARIEPINUS WITH SPECIAL REFERENCES TO THE ASSOCIATED PATHOLOGICAL AND CLINICOPATHOLOGICAL CHANGES

(With 2 Tables and 12 Figures)

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تأثير الإصابة بديدان الدم الطفيلية (مرض السانجو ينيكولا) على أسماك القرموط الأفريقي مع الإشارة إلى التغيرات الباثولوجية والباثولوجية الإكلينيكية الناتجة عن هذه الإصابة

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ديدان السانجوينيكولا التي تصيب الأسماك المستزرعة والأسماك التي تعيش في مياه النيل والترع والمصارف أصبحت أهم وأخطر ديدان الدم الطفيلية كالتي تسبب تحطيم لكل الأعضاء الداخلية للأسماك المصابة مثل أسماك القرموط الإفريقي حيث تم عمل فحص للخياشيم والقلب والكلي والكبد والطحال فوجدت ديدان السانجوينيكولا في القلب. وبالفحص الطفيلي لأسماك القرموط الأفريقي كانت نسبة الإصابة ٦٣,٣٣% بديدان السانجوينيكولا. وبعد الفحص المعملي لأسماك القرموط الأفريقي تبين الأسماك المصابة تسبح ببطيء وغير منتظمة في حركاتها بالإضافة لوجود أنيميا وتغير لون الخياشيم إلي اللون الباهت ولذلك فقد وجدت تغيرات باثولوجية لأنسجة الأسماك المصابة بديدان السانجوينيكولا فبالنسبة للتغيرات في الخياشيم لوحظ موت خلايا نسيج رقائق الخياشيم مع احتقان في الأوعية الدموية الخيشومية مع وجود تورم وإرتشاح وتجمعات كثيرة لخلايا الدم البيضاء وذلك في جانب واحد من القوس الخيشومي أما القلب فقد أصيب بتضخم وإرتشاح مع تجمع لخلايا الدم البيضاء بين أنسجة القلب مع وجود التهاب في عضلة القلب. التغيرات الباثولوجية في الكبد ترى تضخم مع إرتشاح في الأوعية الدموي لجدار الكبد مع تجمع للخلايا الليمفاوية مع تحلل وتكسير في خلايا الكبد ولوحظ تجمع دموي في خلايا الكبد والبنكرياس وتجمع خلايا الميكروفاج مع وجود تتكرز في خلايا الكبد. والتغيرات الباثولوجية التي حدثت للطحال تكمن في انعدام الخلايا المصنعة لخلايا الدم والخلايا الليمفاوية. كما لوحظ تغيرات في أنسجة الكلية الخلفية وتشمل بقع من الأنزفة مع تآكل في العناصر الأساسية التي تتركب منها الكلي من الخلايا المجمعة والمرشحة للدم وكذلك الخلايا الإخراجية. تم تقييم تأثير السانجوينيكولا على أسماك القرموط الإفريقي المصابة والسليمة من خلال صورة الدم وتشمل نسبة الهيموجلوبين وعدد خلايا الدم الحمراء والبيضاء. هذا بالإضافة للتحاليل البيوكيميائية لسيرم الدم وتشمل قياس

نسبة البروتين الكلي ووظائف الكبد والكلي. وأثبتت التحاليل وجود نقص معنوي في تركيز الهيموجلوبين وخلايا الدم الحمراء وحجم الخلايا المضغوطة ووجود زيادة معنوية في كرات الدم البيضاء. وكذلك وجود نقص معنوي في البروتين الكلي لسيرم الدم مع زيادة ملحوظة في وظائف الكلي والكبد لسيرم الدم في المجموعة المصابة عن المجموعة السليمة. وأوضحت النتائج أيضا ارتفاع نسبة خلايا الدم البيضاء ارتفاعا ملحوظا في الأسماك المصابة نظراً لزيادة الأجسام المضادة. أثبتت النتائج أن علاج أسماك القرموط الإفريقي المصابة السانجوينيكولا بدواء (بارازكونتال) درونسيت تحسنت حالة الأسماك فقد تحسنت صورة الدم فزادت نسبة الهيموجلوبين وعدد كرات الدم الحمراء وحجم الخلايا المضغوطة وانخفض العدد الكلي لكرات الدم البيضاء لمستوى المجموعة السليمة وأيضا تحسنت القياسات البيوكيميائية لكل من البروتين الكلي ووظائف الكبد والكلي وعادت إلى ما يقرب من مستواها الطبيعي.

SUMMARY

Sanguinicola species become very important dangerous blood parasites, infested culture wild fishes and caused damge for or yours of infested fish species specially *CLarias gariepinus*. The examination of gills, heart, kidney, liver and spleen revealed that present live blood fluke in some organs e.g. heart. The parasitological examination for *Clarias gariepinus* revealed that the prevelance of *Sanguinicola species* infestation was (63.33%). The clinical signs of examined infested fish showed that fish swim slowly and listlessly, anaemic and gills of infested fish were pale in colour. The histopathological changes in gills of infested *Clarias gariepinus* with *Sanguinicola sp.* showed unilateral sloughing of secondary lamellae and congested branchial blood vessels with presence of edema and intense leukocytic aggregation in gill arch. In heart present intermuscular edema, leukocytic infiltration among degenerated muscle fibers and intense hyaline degeneration or myolsis among muscle fiber. In liver showed edematous vascular wall, lymphocytic infiltration and degeneration in hepatic cells. In hepatopancreas present degranulation, hemorrhages and melanomacrophage aggregations with diffuse necrosis of hepatic parenchyma. In spleen showed depleted haemopiotic and lymphoid elements. In posterior kidney revealed focal hemorrhagic areas, hyaline degeneration in tubular nephrosis and contracted glomerular tufts. Effect *Sanguinicola sp.* infection on *Clarias gariepinus* was evaluated blood haemogram including total RBCs count, total leucocytic count, packed cell volume (PCV) and hemoglobin concentration. Also serum analysis of biochemical parameters including total protein and liver and kidney functions. The total RBCs count, hemoglobin concentration and PCV value were significantly decreased but total leucocytic count were very highly significantly increased due to increase in antibodies levels. The

total proteins were very highly significantly decreased. Serum AST and S.ALT showed high significant and significant increase respectively and serum creatinine were highly increased significantly. The treatment trial was held by using (Praziquantel - Bayer) Droncit in 10 mg/L for 1 hour for 4 days. Blood analysis of fish free from blood fluke (*Sanguinicola sp.*) revealed increase highly significantly in total RBCs count, hemoglobin concentration and packed cell volume. Total protein and total WBCs count were very highly decreased in compared with infested fish.

Key words: *Fish, Clarias gariepinus, blood fluke, sanguinicolosis.*

INTRODUCTION

Parasites that belong to the trematoda (sucking worms) are often to be found in fish. The flattened sucking worms that are parasitic in internal organs of fish have one or two sucking discs. In the blood of fishes several species of the real blood worm *Sanguinicola* may be found as parasites. *Sanguinicola sp.* is most important fish parasite among the expanding aquaculture industry especially in tuna fish, (Colquitt *et al.*, 2001). The adult worms as living in the fish do not possess suckers. Instead they swim actively through the blood by waving movements of their body. They occur most abundantly in the heart and in the larger blood vessels of the gills. The eggs of the worm are transported by blood stream to capillaries of gills, kidneys, heart, liver and other organs. Also larva (miracidia) hatch from the eggs and bore through the wall of capillary of gills to enter lamnaied snails as intermediate host in water then develop into forked cercariae, where they penetrate the final host of fish through gill sheets and weaker parts of the skin. The few adult worms don't harm the fish, but the large number of cercaria in blood may kill fish (Kirk and Lewis, 1992). Blood flukes are known to cause significant pathology in several other marinculture species, such as cultured sea bass, *Lates calcarifer* (Bloch), in Malaysia (Herbert *et al.*, 1995) and have caused mass mortality in Japanese amberjack, *Seriola dmerili* (Risso), juveniles as reported by Ogawa & Fukndom (1993). Eggs lodging in the viscera or connective tissue are encapsulated by the inflammatory granulomatous response of the fish host. Pathological changes caused by flukes can severely damage fish (Kirk and Lewis, 1992).

The separation and characterization of serum protein components of infected and non infected fish can help us to evaluate the host parasites relationship (Woo, 1992).

For prevention of blood fluke infection in farmed fish is the destruction of intermediate host snail and elimination of adult flukes from infected fish (Paperna, 1955).

The aim of study included, prevalence of sanguinicola species in clarias gariepinus, clinical signs of infestation with parasites and evaluate the haematological, biochemical and histopathological changes from natural infection with Sanguinicola sp. and treatment.

MATERIALS and METHODS

1- Examined fish:

Sixty fishes (*Clarias gariepinus*) were collected from River Nile in Dakahlia governorate. Fish were transported alive put in glass aquarium and clinical then were detected.

2- Collected blood samples:

Fresh blood samples were collected from caudal artery of both infected and non infected fish in tubes contain EDTA and tubes without EDTA.

a- Haematological examination:

Blood samples used for determination of total erythrocytic count (RBCs), hemoglobin concentration (Hb), total leucoytic count (WBCS), packed cell volume (PCV) according to Stoskopf, (1993).

b- Biochemical examination:

Serum samples were obtained from infected and non infected fish by centrifugation of blood samples and kept in refrigerator at -20°C until used. Total protein, aseparate aminotransferase (A.AST), alanine aminotransferase (S.ALT) and creatinine were determined by using commercial diagnostic kits from Merieux Laboratory Reagents and Products, France.

3- Parasitological examination:

The parasitological examination for heart and its blood vessels after flushed with PBS and the washing examined under dissecting microscope. Also gills and kidney were examined. The isolated blood parasites trematodes were preserved in formalin (10%) and stained with aceto acid carmine stain then identified according to Schmidt (1993).

4- Histopathological studies:

The gills, heart, liver, spleen and kidney of infested fish were taken, preserved in 10% buffered natural formalin and prepared for histopathological examination according to Roberts (1989).

5- Treatment trial:

Seven (7) of alive *C. gariepinus* which proved to be heavily infested with *Sanguinicola sp.* after parasitological examination and

other group was healthy and free from Sanguinicola sp. infestation were used control group. Fish can be kept in glass aquaria with dechlorinated water at 22°C ± 0.5. Droncit (praziquantel- Bayer) was used for treatment of infested fish in dose 10mg/liter for 1 hour for 3 days by bath method (Paperra, 1995; El-Khatib and Elias 2000). Blood samples were taken after 3 weeks from treatment for parasitological, haematological and biochemical examination.

6- Statistical analysis:

The *t*-student test was used for statistical analysis for obtained data according to Petrie and Watson (1999).

RESULTS

The prevalence of infestation with Sanguincola sp.was 38 from 60 examined fish (63.33%). The clinical signs of infested fish showed that fish swim slowly and listlessly, anaemic and gills pale in colour. The examined heart contains live adult flukes (Figs. 1 & 2).

Histopathological examination revealed that congested branchial blood vessels accompanied with unilateral sloughing of secondary lamellae and hypertrophy and fusion of secondary lamellar epithelium in the opposite side cloud be seen (Fig. 3). The gill arch showed edema, intense leukocytic aggregations and hemorrhages (Fig. 4). Surface epithelium of the gill rocker showed metaplsia to goblet cells. Some parasitic elements could be seen within gill arches. The histopathological changes in the heart revealed intermuscular edema and few leukocytic infiltration among degenerated muscle fibers were seen (Fig. 5). Some muscle bundles suffered from intense hyaline degeneration or myolysis (Fig. 6).

Also the pathological changes in liver showed edematous vascular wall with wild perivascular lymphocytic infiltration and degeneration changes in the hepatic cells were seen (Fig. 7). Hepatopancreas showed inactivation or degranulation and necrotic changes in the surrounding hepatic cells beside presence of melano macrophage aggregations (Fig. 8). Periductula fibrosis and congested blood vessels were encountered. Diffuse necrosis of the hepatic parenchyma which usually infiltrated with lymphocytes could be seen (Fig. 9). Also spleen showed depleted haemopiotic and lymphoid elements and congested elpsoides with presence of humerous melanomacrophage centers were the common splenic lesions (Fig. 10). The pathologic changes of posterior kidney revealed that focal hemorrhagic areas with active hemopoietic elements were the

predominant changes (Fig. 11), tubular nephrosis mainly hyaline degenerations and contracted glomerular tufts were the predominants tubular change (Fig. 12).

Haemogram picture of infected group of *Clarias gariepinus* revealed that TRBCs, Hb and PCV values decreased highly significantly ($P<0.01$) on contrary TWBCs showed very highly significantly ($P<0.01$) increase were shown in Table (1). The biochemical parameters of blood serum evaluation showed that the total protein value decreased very highly significant ($P<0.001$), serum aseparate aminotransferase (A.AST) increased significantly ($P<0.05$), serum alanine aminotransferase (S.ALT) and creatinine also showed high significant increase at ($P<0.01$) over the level of control group as in Table (2).

Treatment trial:

After treatment of infected *Clarias gariepinus* group and free from *Sanguinicola sp.* infestation. All haematological and serum biochemical parameters showed marked improvement towards the normal levels as illustrated in Tables (1 & 2).

Table 1: Mean values of some haematological parameters of the control group and infected group of *Clarias gariepinus* with *Sanguinicola sp.*

Item / Parameters	Control group	Infected group	
		Before treatment	After treatment
TRBCs (x 10^6/mm^3)	3.05 ± 0.13	2.20 ± 0.15**	3.21 ± 0.21
Hb (G/dl)	9.10 ± 0.37	6.65 ± 51**	8.98 ± 0.56
PCV (%)	23.20 ± 0.79	18.09 ± 1.01**	22.77 ± 0.80
TWBCs (x 10^3/mm^3)	26.34 ± 1.01	32.59 ± 0.96***	27.11 ± 0.88

** Highly significant at $P < 0.01$

*** Very highly significant at $P < 0.001$

Table 2: Mean values of total protein, liver and kidney functions of the control group and infected group of Clarias gariepinus with Sanguinicola sp.

Item / Parameters	Control group	Infected group	
		Before treatment	After treatment
Total protein (G/dl)	4.75 ± 0.22	2.69 ± 0.19***	4.59 ± 0.29
S.AST (Iu/l)	265.23 ± 2.02	272.15 ± 2.24**	266.99 ± 1.72NS
S.ALT (Iu/l)	6.78 ± 0.14	7.88 ± 0.23**	6.81 ± 0.17NS
S. creatinine (mg/dl)	1.68 ± 0.10	2.28 ± 0.11**	1.74 ± 0.13NS

* Significant at ($P < 0.05$)

** Highly significant at ($P < 0.01$)

*** Very highly significant at ($P < 0.001$)

NS: Non significant.

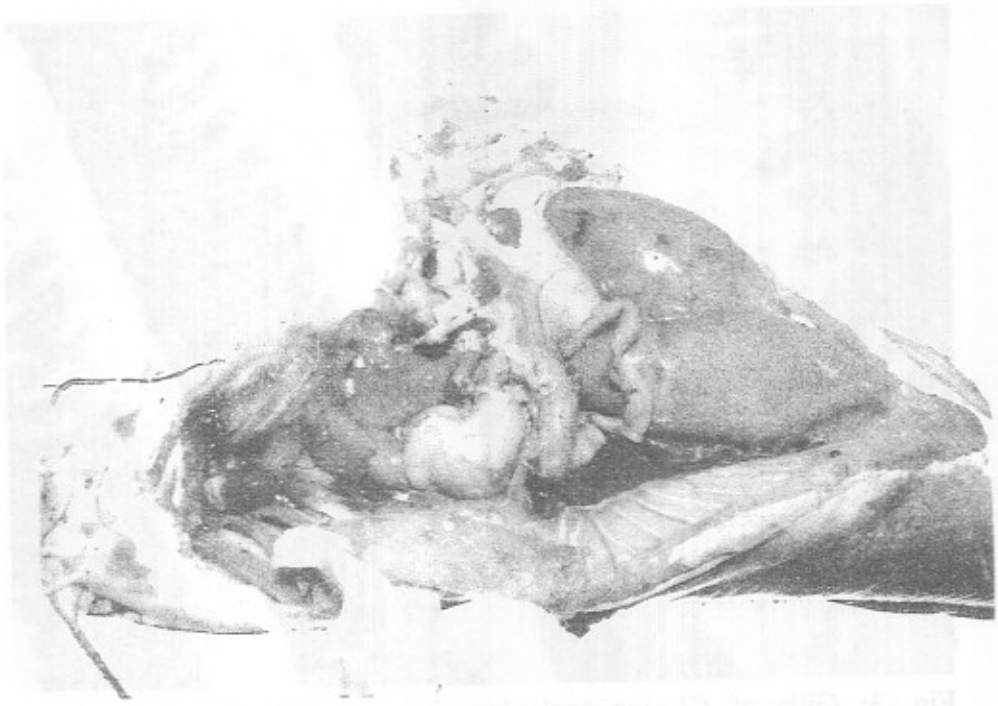

Fig. 1: *Clarias gariepinus* infected with *Sanguinicola* sp. showing gills was pale in colour

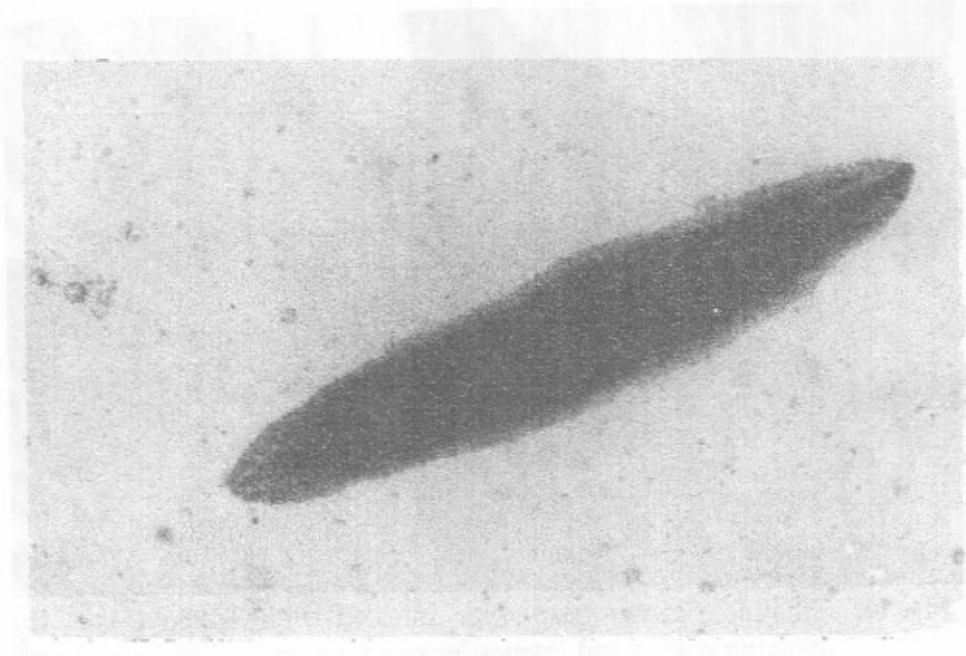

Fig. 2: *Sanguinicola sp.* x 40

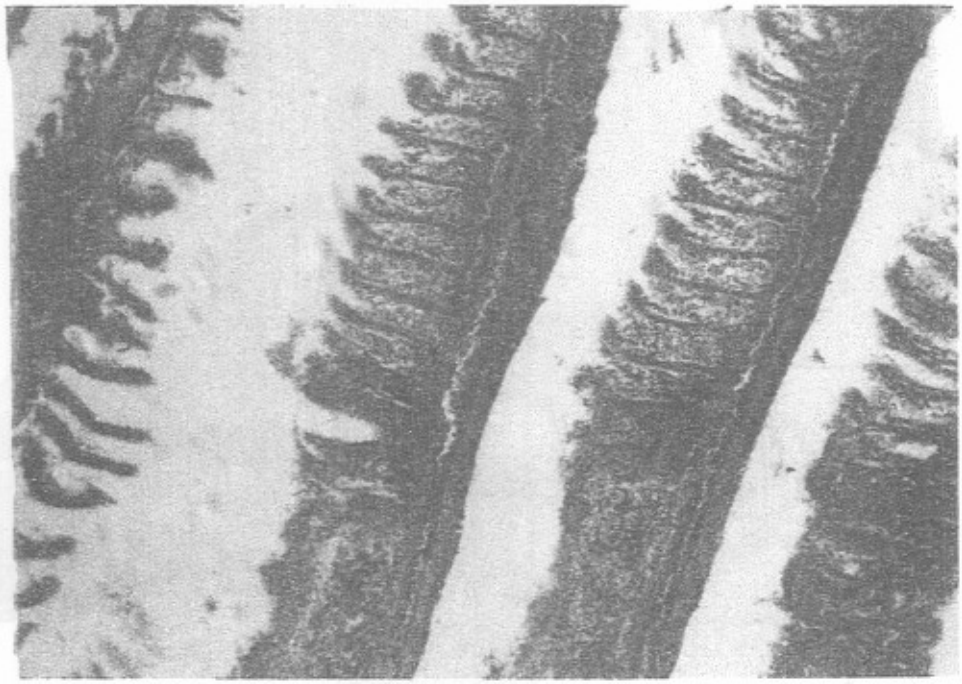

Fig. 3: Gills of *Clarias gariepinus* infested with *Sanguinicola sp.* showing unilateral sloughing of secondary lamellae and congested branchial blood vessels (H & E x 150).

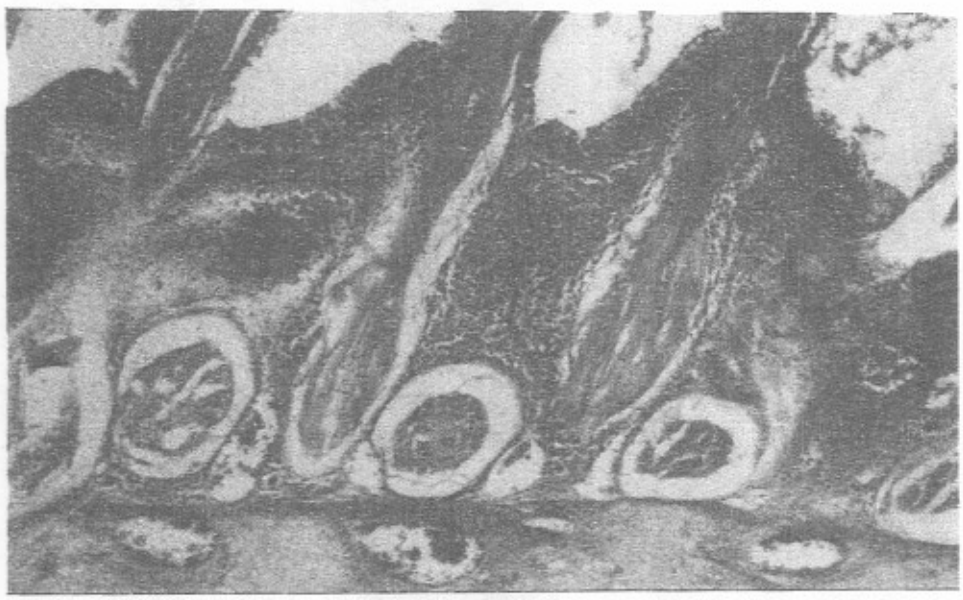

Fig. 4: Gills of *Clarias gariepinus* infested with *Sanguinicola sp.* showing edema and intenes leukocytic aggregations in gill arch (H & E x 300)

Fig. 5: Heart of *Clarias gariepinus* infested with *Sanguinicola sp.* showing edematous myocardial muscles (H & E x 150)

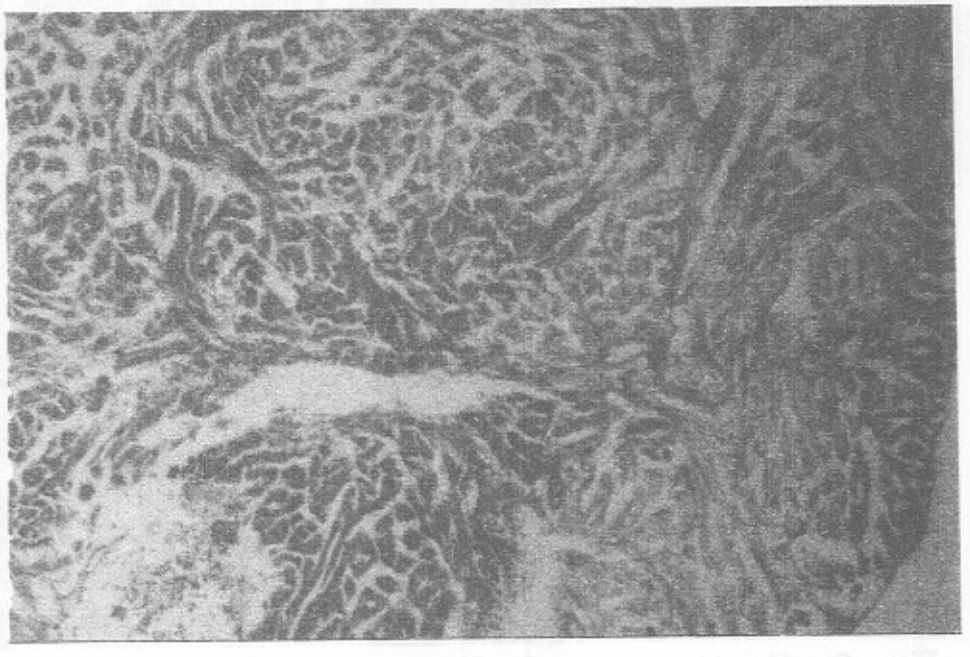

Fig. 6: Heart of *Clarias gariepinus* infested with *Sanguinicola sp.* showing intense Zenkers degeneration or myolysis of muscle fibers (H & E x 300)

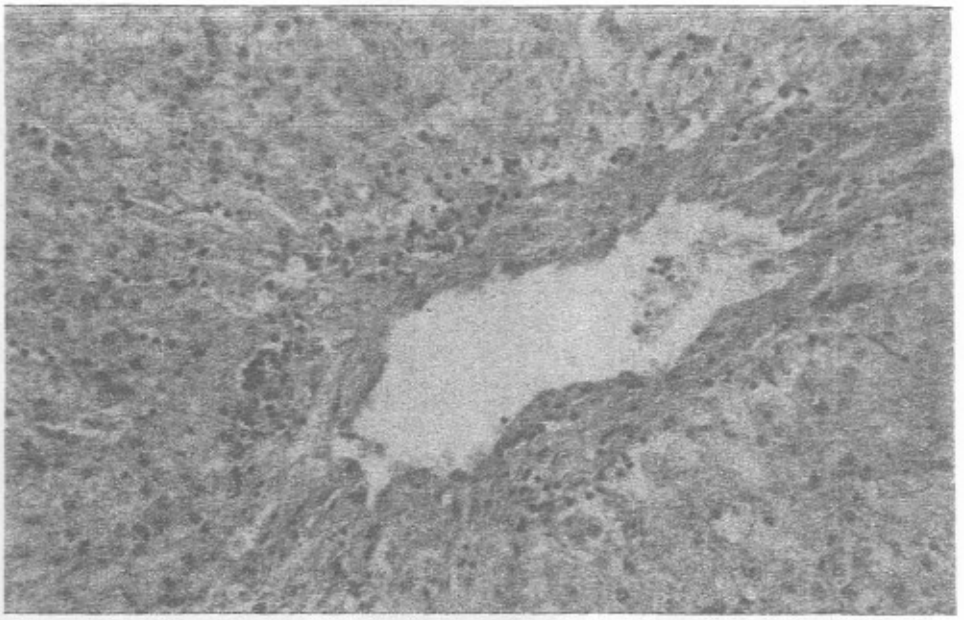

Fig. 7: Liver of *Clarias gariepinus* infested with *Sanguinicola sp.* showing adematous vascular wall and degenerated hepatic cells (H & E x 150)

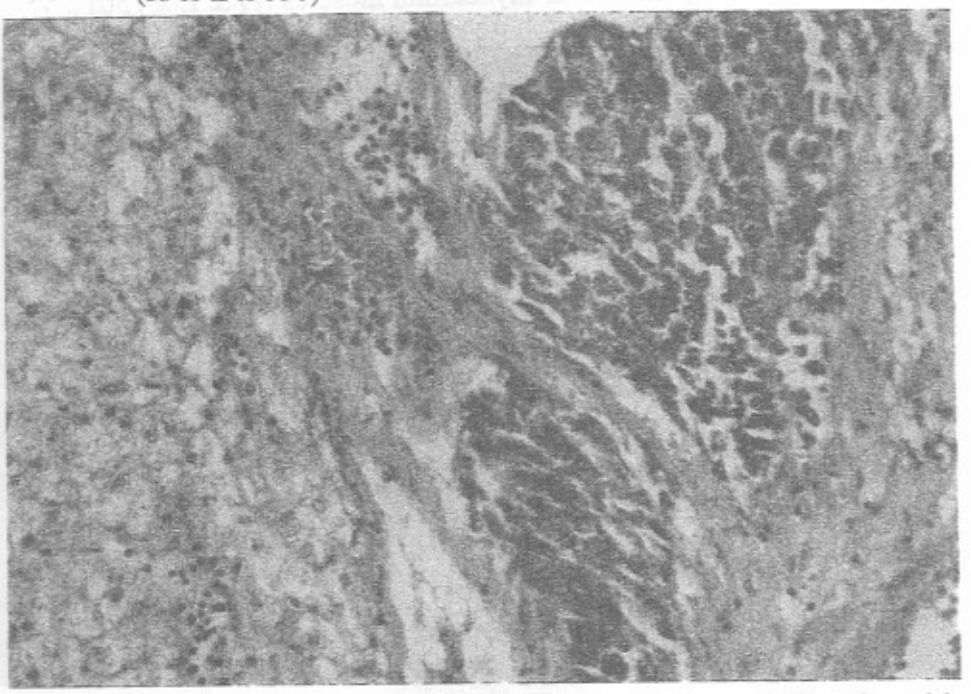

Fig. 8: Hepatopancreas of *Clarias gariepinus* infested with *Sanguinicola sp.* showing degeneration and inactivation (H & E x 300)

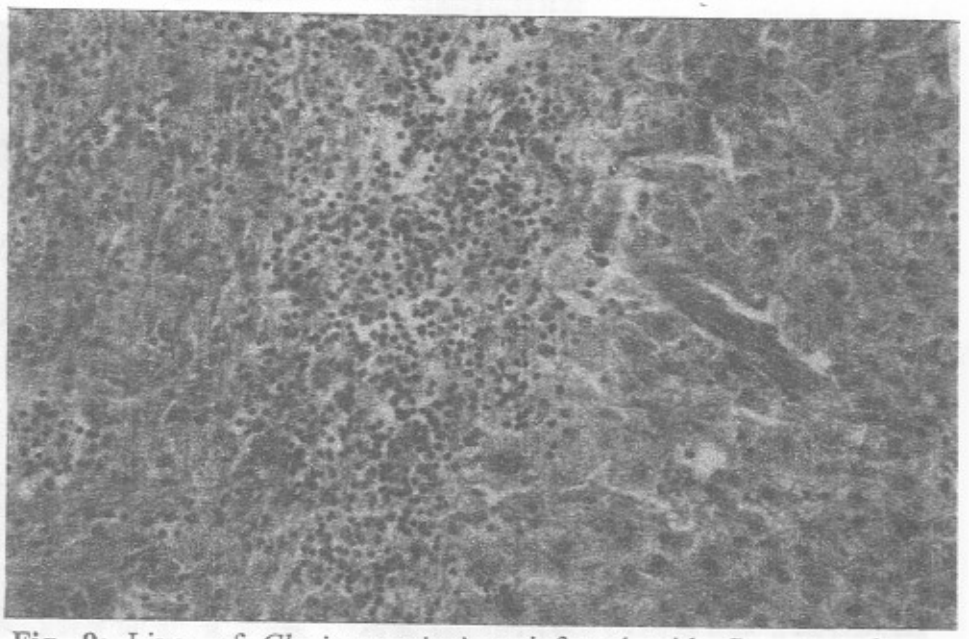

Fig. 9: Liver of *Clarias gariepinus* infested with *Sanguinicola sp.* showing diffuse hepatic necrosis infiltrated with lymphocytes (H & E x 300)

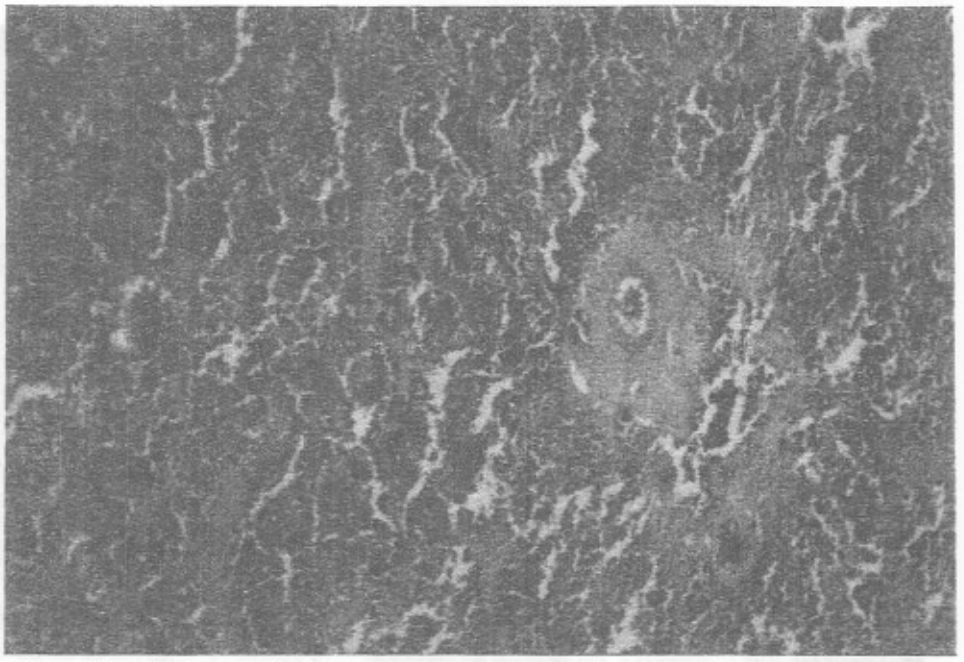

Fig. 10: Spleen of *Clarias gariepinus* infested with *Sanguinicol sp.* showing depleted hemopoietic and lymphoid elements (H & E x 150)

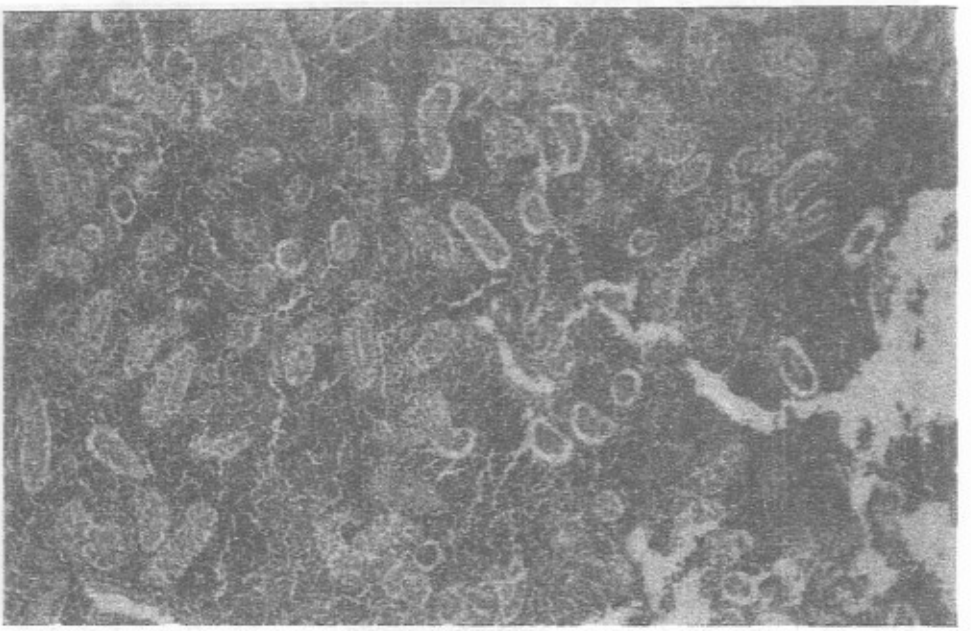

Fig. 11: Posterior kidney of *Clarias gariepinus* infested with *Sanguinicola sp.* showing focal hemorrhages and active haemopiotic elements (H & E x 150)

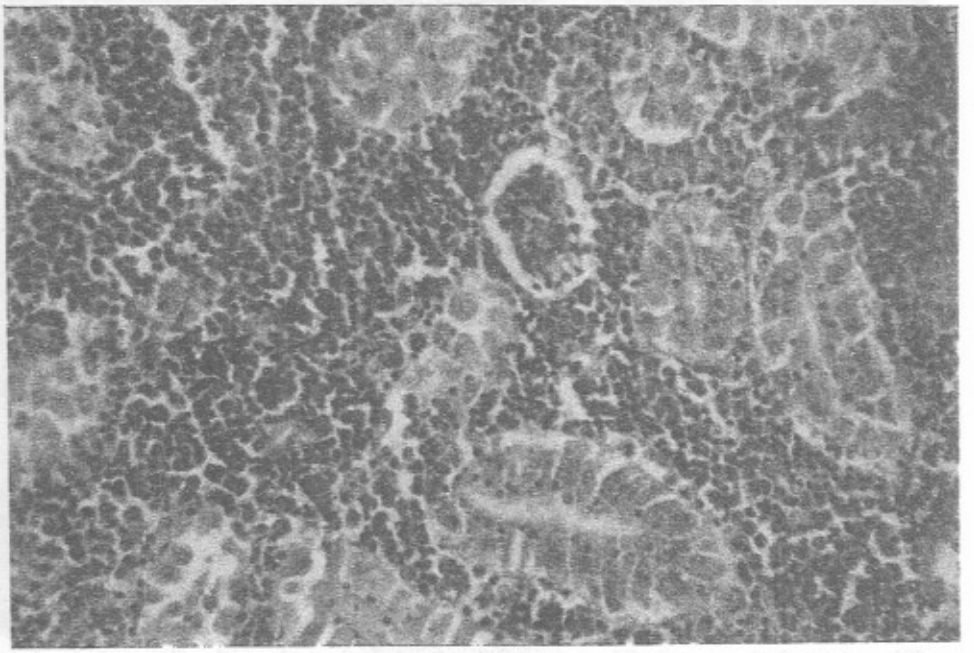

Fig. 12: Posterior kidney of *Clarias gariepinus* infested with *Sanguinicola sp.* showing tubular nephrosis and contracted glamerular tufts (H & E x 300)

DISCUSSION

Blood flukes are becoming recognized as important fish parasites in the aquaculture industry. The recorded number of blood fluke species and fish hosts is increasing, as recorded by Smith (1997).

In the present study, the prevalence of infestation with *Sangunicola* species in *Clarias gariepinus* was 36.33% and clinical signs of infested fish included gills pale in colour and fish anaemic. This result was agreed with El Khatib and Elias (2003).

The histopathological study in gills revealed that congestion branchial blood vessels, unilateral sloughing of secondary lamellae, hypertrophy and fusion of secondary lamellar epithelium in opposite side and edema in gill arch with leukocytic aggregation and hemorrhages. Surface epithelium of gill rocker showed metaplasia to goblet cells and some parasitic elements could be seen within gill arches which may be due to damage of gills tissues and obstruction of blood vessels caused reducing in blood circulation, hemorrhage and inflammatory responses lead to respiratory manifestation during invasion and migration of blood flukes and miracidia through gills of fish. These results agree with Kirk and Lewis, (1998) and Colquitt *et al.* (2001).

The pathological changes in heart of infected fish were intermuscular edema and few leukocytic infiltration among degenerated muscle fibers and some muscle bundles suffered from intense hyaline degeneration or myolysis. These results were go parallel with the findings of Colquitt *et al.* (2001) were reported that fibrotic tissue and myocardial hypertrophy could stroke volume, thus requiring an increase in heart rate cause stress in fish especially during times of anoxia/hypoxia and increase metabolic oxygen demand.

Also the pathological changes in liver showed edematous vascular wall with mild perivasculor lymphocytic infiltration and degenerative changes in hepatic cells, also hepatopancreas showed inactivation or degranulation hemorrhages and necrotic changes in the surrounding hepatic cells beside presence of melanomacrophage aggregation. The periductula fibrosis and congested blood vessels were encountered. Diffuse necrosis of hepatic parenchyma which usually infiltrated with lymphocytes. These changes due to parasitic invasion were hepatic cells necrosis and lymphocytes presence due to reactive hepatitis in response to systemic infection and toxic material released from invasive parasites lead to serious damage hepatic parenchyma effect on metabolism of protein, carbohydrate and lipid. The

hemorrhages and melanomacrophage due to haemolytic anaemia and ferrous iron stored in melanomacrophages of haemopoietic tissue of liver. These results agreed with Roberts, (1989).

The histopathological changes in spleen showed depleted haemopioetic and lymphoid elements and congested elpsoides with presence of numerous melanomacrophage centers were the common splenic lesion, these results agreed with Richards *et al.* (1994) were recorded that ultrastructural changes cells of cyprinus carpio spleen, parasitism induced significant reductions of erythrocytes, neutrophils and eosinophils and significant increases of thrombocytes and macrophages.

In other hands, the histopathological changes in kidney showed that the posterior kidney containing focal hemorrhagic areas with active haemopiotic elements were the predominant changes, tubular nephrosis mainly hyaline degeneration and contracted glomerular tufts were the predominants tubular changes beside melanomacrophage, these results agreed with Richards *et al.* (1994) and Kirk and Lewis (1998).

The haemogram picture of infested fish with *Sanhuinicola sp.* were present anaemia due to loss of blood during miracidia liberate from gill tissue to swim in water and blood flukes excreted toxic materials in blood of infested fish were caused changes in blood contents including decreased in RBCS count and hemoglobin level and increase WBCS count these results were agreement with Williams (1967) and Gomez-Bautista and Simon-Martin, (1987) were recorded that haematocrite and hemoglobin levels had dropped in *Rutilus arcasi* (Cypyindidae) infected with *Sanguinicola sp.* Serum total protein value were very highly decreased due to liver damage of infected fish, these results agreed with Ezz- Edin & Mousa (1998) and El-Khatib and Elias, (2002) but after treatment, total protein value were increased sera analysis of liver enzymes level of infected group regarded significant increase in S.AST and highly significant increase in S.ALT, also serum creatinine of infected group showed highly significant increase due to liver and kidney dysfunction during *Sanguinicola sp.* infection (Stoskopf, 1993).

The haematological and biochemical parameters were returned to normal levels after treatment of infested *Clarias gariepinus* with Droncit and become free blood parasite (*Sanguincola sp.*).

Finally, from this study it was concluded that, blood parasites (*Sanguinicola sp.*) have dangerous effect on health of *Clarias gariepinus* includes haematological, biochemical parameters and histopathological changes.

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