Clinicopathological Studies on Blood of African catfish (*Clarias gariepinus*) Infected with *Pseudomonas florescence*

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Abstract

The present study was planned to investigate the clinicoplathological and histopathological changes on the blood of catfish (*Clarias gariepinus*) infected with *Pseudomonas florescence*. A total number of 240 catfish were collected from Ismailia channel and subdivided to 4 groups 60 for each: the control group, infected group, β-glucan group and β-glucan infected group. The blood samples were taken at 1, 3 and 5 weeks for hematological and biochemical examinations. Tissue samples from spleen, liver, kidney and gills were taken for histopathological techniques. The hematological results revealed significant decrease of RBCs count, Hb concentration and PCV% with significant increase of T.L.C, heterophils and lymphocytes of the infected group compared to the control group. The β-glucan groups showed significant increase of RBCs count, Hb and PCV% with significant increase of T.L.C, heterophils, lymphocytes and monocytes. Serum biochemical results showed marked elevation in ALT, AST, urea, creatinine, uric acid and glucose of the infected group. While, β-glucan improved the liver function, kidney function tests and glucose before and after the infection compared to the control group. The challenge test revealed a significant lower mortality percentage in the group received β-glucan supplemented diets. Histopathologically, marked degenerative changes and necrosis were evident in the infected group. While, β-glucan groups showed hyperactivity of melenomacrophage centers of haemopiotic organs. It could be concluded that β-glucan acts as a dietary supplement.

Introduction

Aquaculture production has increased from representing 9% of the fisheries resources in 1980 to a current 43%, and it is thought that the production will need to be double in the next 25 years. ***(FAO,***

2010). In recent years, the production of catfish has suffered massive financial losses due to pathogen spread and breakouts. Pseudomonas septicemia is one of the most prevalent fish diseases in aquaculture due to its ubiquitous nature in aquaculture environment ***(Fernandez et al, 1990)***.

The long-term administration of antibiotic growth promoters, AGPs, in aquafeeds creates an optimal environment to enable antibiotic resistance genes to multiply ***(Lu¨cksta¨dt, 2006).*** Vaccination programs are the second control measures used in recent day fish farms, but the vaccine production is expensive and lengthy process ***(Heppel and Davis, 2000)***. The development of non-antibiotic and environmentally friendly agents is one of the key factors for health management in aquaculture ***(Bidhan et al, 2014)***. Probiotics, according to the currently adopted definition, are live microorganisms that when administered in adequate amounts confer a health benefit on the host ***(FAO/WHO, 2001).*** The success of probiotics, has led to foundation of other concepts like "Prebiotics". The most recent definition of prebiotics was agreed by ***(Gibson et al, 2011)*** which is "A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.The most promising group of prebiotic are the β-1,3/1,6-glucans, a soluble carbohydrates from the cell walls of yeast *Saccharomyces cerevisiae*, because they have a well-defined chemical structure and mode of action on the immune system ***(Raa, 2000).***

This study was designed to investigate the effect of *Pseudomonas florescence* on blood of catfish (*Clarias gariepinus*) and their associated clinicopathological and histopathological changes.

Materials and Methods

A total number of (240) apparently healthy African catfish (*Clarias gariepinus*) were randomly collected alive from Ismailia channel with an average body weight 150 ± 25gm. They were transported in a natural water tank to the lab. at Fish Diseases and Management Department, Faculty of Veterinary Medicine, Suez Canal University and kept for two weeks for acclimation in glass aquaria and the water was renewed daily. The temperature of 25°C ± 1°C was adjusted thermostatically by using heater and continuous aeration using electric air pumping compressors.

Two rations were used in this study. The control ration consisted of commercial floating pellets consisting of 25-30% crude protein, 5.5% crude fat, 3.4% crude fiber, vitamins and mineral premix obtained from (Zoo-control Company, Egypt). The second ration, contained the basal

commercial ration, supplemented with β-1, 3 glucan extracted from the cell wall of yeast (*S. cerevisiae*) at a concentration of 1g/ 1 kg ration (obtained from Anhui ZhengZheng Biology Technology Co., Ltd.). The fish were divided into four groups (60 for each), then each group subdivided into 3 subgroups (20 fish/ aquarium) as follows:

Control group: fish fed on basal diet non- infected non-supplemented.

Infected group: fish fed on basal diet and infected by injection i.p. with 1 ml ($1x10^7$ CFU/fish) of well identified virulent strain of *P. florescence*.

β-glucan group: fish fed on basal diet supplemented with (1g β-1,3 glucan/kg diet) for 5 weeks.

β-glucan infected group: fish fed on basal diet supplemented with (1g β-1,3 glucan/kg diet) and then at the 5^{th} week infected with 1 ml ($1x10^7$ CFU/fish) of well identified virulent strain of *P. florescence*

Clinical signs and postmortem findings of the infected fish were performed according to the methods given by ***Schaperclaus et al (1992)*** and ***Fergnsen (1989)*** respectively.

Blood sampling:

Two blood samples from each fish were collected from the caudal blood vessels of fish. One sample was taken in a tube containing EDTA and used as a whole blood for hematological picture. The second blood sample was taken in a clean centrifuge tube, and then centrifuged at 3000 r.p.m for 5 minutes for separation of sera that kept at -20 till analysis of serum biochemical parameters.

Haematological and biochemical tests:

a) Hb concentration, RBCs and WBCs were determined according to ***Stoskopf (1993)*** and ***Natt and Herrick (1952)*** respectively. PCV % was determined according to ***Decei and Lewis (1991),*** the blood indices were determined according to ***Coles (1986)*** and the differential leukocytic count was examined according to ***Thrall (2004).***

b) Serum AST and ALT were estimated according to ***Bakker et al (2007).*** Urea, creatinine and uric acid were determined according to ***Löhr et al (2009)*** and ***Staples et al (2010)*** respectively and serum glucose was determined according to ***Tietz, (2006).***

Histopathological techniques:-

Tissue specimens from the different organs (spleen, liver, anterior kidney and gills) of infected and β-glucan infected groups were dissected and immediately fixed in 10% formalin solution for 48-72 hrs. The histological techniques were carried according to ***Drury and Willington (1980).***

Statistical analysis

The obtained data were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS program version 16 (SPSS, Richmond, VA, USA) as described by ***Dytham (1999).***

Results and Discussion

The clinical signs of the experimentally infected catfish (*Clarias gariepinus*) after injection with *Pseudomonas florescence* revealed exophthalmia, petechiae on the body surface and hemorrhagic spots at the base of fins, fin rots and skin ulceration with excessive mucus all over the body surfaces. The postmortem findings showed signs of septicemia with congestion of the internal organs, splenomegaly, enlarged and friable kidney and the liver revealed pale color in some cases or deep brown with hemorrhagic patches and necrotic foci on its surface. Ascites, enlarged and distended gall bladder. These signs were nearly similar to the result recorded by ***Hanna et al (2014) and Saad et al (2014).***The clinical signs and P.M. lesions may be attributed to the effect of proteolytic enzymes and exotoxins production of *P. florescenc*e that affected the endothelial lining of the blood vessels and the tissues ***(Pandey, 2014).***

Concerning the haemogram in table (1), it was shown that RBCs, Hb concentration, PCV % and MCHC were significantly ($p < 0.05$) decreased in the infected group when compared with the other three groups starting from the first week till the 5th week. While MCV was significantly ($p< 0.05$) increased in the infected group when compared with the other three groups during the whole experimental period. Similar results were reported by ***Wafaa (2007) and Saad et al (2014).*** The reduction in the erythrocytic parameters could be attributed to the observed hemorrhagic patches in P.M findings and /or red blood cell lysis. ***Fernandez et al (2003)*** explained that Pseudomonas produces products as hemolysin and proteolytic enzymes which cause lysis and destruction of RBCs and reduce its number and its Hb content. The anaemia was macrocytic hypochromic that indicated by increase MCV and decreased MCHC, probably an indication of RBC swelling and/or a decrease in Hb synthesis ***(Mydeen et al, 2013).*** The histopathological examination of the hematopoietic tissues of the spleen and anterior kidney in photos (a & e) which showed depletion of some lymphoid follicles and severe congestion of blood vessels were in line with these findings. Looking to the results obtained at the 5th week, it was observed that higher significant ($p< 0.05$) increases of RBCs, Hb concentration, PCV % at β-glucan group compared with the all groups. The results were in agreement with those of ***El-Boshy et al (2008) and Sara (2012).*** While, ***Sahan and Duman (2010)*** reported non-significant changes in blood indices of Nile tilapia supplemented with β-1,3glucan diet. The results may be attributed to the hepato–protective and hepato–stimulatory effects of

the β-glucan which lead to the release of erythropoietic factors by the hepatocytes which control erythropoiesis resulting in increase the level of RBCs formation as mentioned by ***Sarma et al (2003)***.

The result of leukogram in table (2) revealed that infected group showed significant (p□ 0.05) increases in T.L.C, heterophils and lymphocytes compared with the other three groups starting from the first week till the 5th week. These results agreed with ***Wafaa (2007) and Lamees (2013)***. The leukocytosis observed in infected group may be due the induction of non-specific defense system and/or phagocytosis and cytotoxic activity. The lymphocytosis may be due to the antigenic stimulation with increased T-lymphocytes by bacterial infection ***(Coles, 1986)*** and ***Paingrahi et al (2005)***. The histopathological findings of leukocytic infiltration in photos (a, c and e) confirms our results. There were leukocytosis (heterophils, lymphocytes and monocytes) in β-glucan and β-glucan infected groups when compared to the control group starting from the 3rd week to the end of the experiment. These results agreed with ***Gado et al (2014)***. The results could be attributed to, β-glucan has specific receptors on the surface of circulating and tissue phagocytes cells (heterophils and monocytes). β-glucan binds to the receptors molecules on the surface of phagocytes and increases their phagocytic activities in engulfing, killing and digesting bacteria and at the same time, they secrete signal molecules (cytokine) which stimulate the formation of new white blood cells ***(Raa, 2000).***

The results of serum aminotransferases in table (3) showed significant ($p < 0.05$) increases in the infected group compared with all group starting from the first week till the 5th week. The elevation of ALT and AST may be attributed to the hepatcellular damage caused by the infection which leads to extensive liberation of these enzymes to the blood circulation ***(Soltan et al, 2008)***.This results were in agreement with ***Lamees (2013) and Saad et al (2014).*** These results were in line with the hisopathological findings of liver in (photo c) of the present study which showed congestion of blood vessels, focal lymphocytic infiltrations, vacular degeneration and necrosis of hepatocytes. A significant ($p < 0.05$) decrease in the serum levels of ALT and AST in β-glucan groups at 3 weeks till the end of the experimental period then they begin to increase but still lower than the control group. These results agreed with that obtained by ***El-Boshy et al (2008)*** *and* ***Sara (2012).*** The lowest values of ALT and AST in β-glucan indicated a normal, positive and beneficial effect of these prebiotics feed additives on the maintenance of the hepatocytes integrity. The normal histopathological pictures of the hepatocytes in photo (d) along with

hyperplasia of melanomacrophage centers of the liver of these groups came in line with the our results.

Regarding the kidney function tests recorded in table (3), there were significant ($p< 0.05$) increases of urea, creatinine and uric acid in the infected group during the whole period of the experiment compared to the all groups that indicated alteration in normal physiology of the kidney due to bacterial toxins ***(Coles, 1986)***. These results agreed with ***Rehulka (2002) and Wafaa (2007)*** who attributed these alterations to the diffuse and necrosis of the renal tubules and depletion of tissue which caused by *P. florescence* infection leading to kidney dysfunction**.** These results came in line with the result of the histopathological examination of the kidney in photo (g) that showed diffuse degeneration and necrosis of renal tubules, congestion of blood vessels and depletion of hematopiotic tissue. Urea is excreted primarily by the gills rather than the kidney ***(Stoskof, 1993)***, so the elevation of urea in our work could be attributed to gill dysfunction. The histopathological examination of the gills in photo (g) showed epithelial desquamation, hyperplasia, vacuolar degeneration, congestion and lymphocytic cell infiltrations in the primary and secondary lamellae. Concerning the effect of β-glucan, there were significant ($p< 0.05$) decreases in the serum levels of urea and uric acid compared to other group from the 3rd week to the end of experiment, this indicating that incorporation of this prebiotic in the diet can improve kidney function. The results were agreed with ***El-Boshy et al (2008).*** On the other hand, the results disagreed with ***Nermeen (2011)*** who recorded marked elevation of creatinine concentration and blood urea nitrogen in fish treated with the whole yeast and prebiotic (MOS). The histopathological findings of kidney in photo (f) of this group that showed hematopiotic tissue and activation of melanomacrophage centers which came in line with the present results

The blood glucose level in table (3) showed a significant ($p< 0.05$) increase in the infected group during the whole period of the experiment compared to the all groups. Similar result was reported by ***Wafaa (2007).*** The increase of blood glucose is characteristic of stress response and stimulation of glycogenolysis as a result of the destructive effect of the bacterial toxins on liver cells ***(Coles, 1986).*** While, the serum glucose level showed non-significant change in the β-glucan supplemented groups compared to the control group, these results agreed with ***El-Boshy et al (2008).*** On the other hand, our result disagreed with ***Gunasundari et al (2013)*** who observed significant increase in the glucose level in clownfish fed on diet supplemented with brewer's yeast.

It could be recommended that, β-glucan has the ability to counteract immunosuppression induced by *Pseudomonas florescence* in catfish to act acts as immunostimulant and disease control. It is recommended as a dietary supplement in order to improve aquaculture production.

Table 1: *Erythrogram (mean value ± SE) in different experimental groups*

groups	Time	RBCs 10^6/µl	Hb gm/dl	PCV %	MCV fl	MCH Pg	MCHC %
Control	**1 week**	2.42 ± 0.06^a	9.62 ± 0.05^a	31.05 +	128.30± 0.28^b	39.71 + 0.44^a	30.99 ± 0.37^a
	3 weeks	2.45 ± 0.01^b	9.51± 0.09^b	32.69 +	133.42 ± 0.68^b	38.79 + 0.34^a	29.10 ± 0.48^a
	5 weeks	2.60 ± 0.03^c	9.35± 0.08^c	29.21 +	112.34 ± 1.50^b	35.95 + 0.64^b	32.01 ± 0.48^a
Infected	**1 week**	1.86 ± 0.02^b	6.72 ± 0.06^b	25.11 +	134.98 ± 0.87^a	36.10± 0.43^b	26.78 ± 0.40^b
	3weeks	1.77 ± 0.02^c	6.46 ± 0.14^c	24.37 +	137.68 ± 0.50^a	36.48 + 0.67^b	26.50 ± 0.47^b
	5 weeks	1.96 ± 0.02^d	7.33 ± 0.13^d	26.07 +	133.61 ± 0.68^a	37.39 + 0.33^a	28.10 ± 0.22^b
β-glucan	**1 week**	2.50 ± 0.05^a	9.64 ± 0.03^a	31.34 +	125.35 ± 1.94^b	38.51± 0.72^a	30.77 ± 0.15^a
	3 weeks	2.58 ± 0.03^a	9.90 ± 0.06^a	34.55 +	133.90 ± 0.35^b	38.36 + 0.42^a	28.65 ± 0.43^a
	5 weeks	2.95 ± 0.03^a	10.99 +	33.65 +	114.06 ± 1.90^b	37.25 + 0.79^a	32.66 ± 0.24^a
β-glucan infected	**1 week**	2.51 ± 0.06^a	9.65 ± 0.03^a	31.47 +	125.36 ± 1.82^b	38.43 + 0.84^a	30.65 ± 0.35^a
	3 weeks	2.57 ± 0.02^a	9.99 ± 0.04^a	34.40 +	134.81± 0.42^b	38.87 + 0.40^a	29.04 ± 0.27^a
	5 weeks	2.83 ± 0.03^b	10.68 +	32.38	114.41± 1.8^b	37.73 + 0.81^a	32.97 ± 0.49^a

Superscript with different letters in the same colum at the same week are significant at ($p < 0.05$)

Table 2: *Leukogram (mean value ± SE) in different experimental groups*

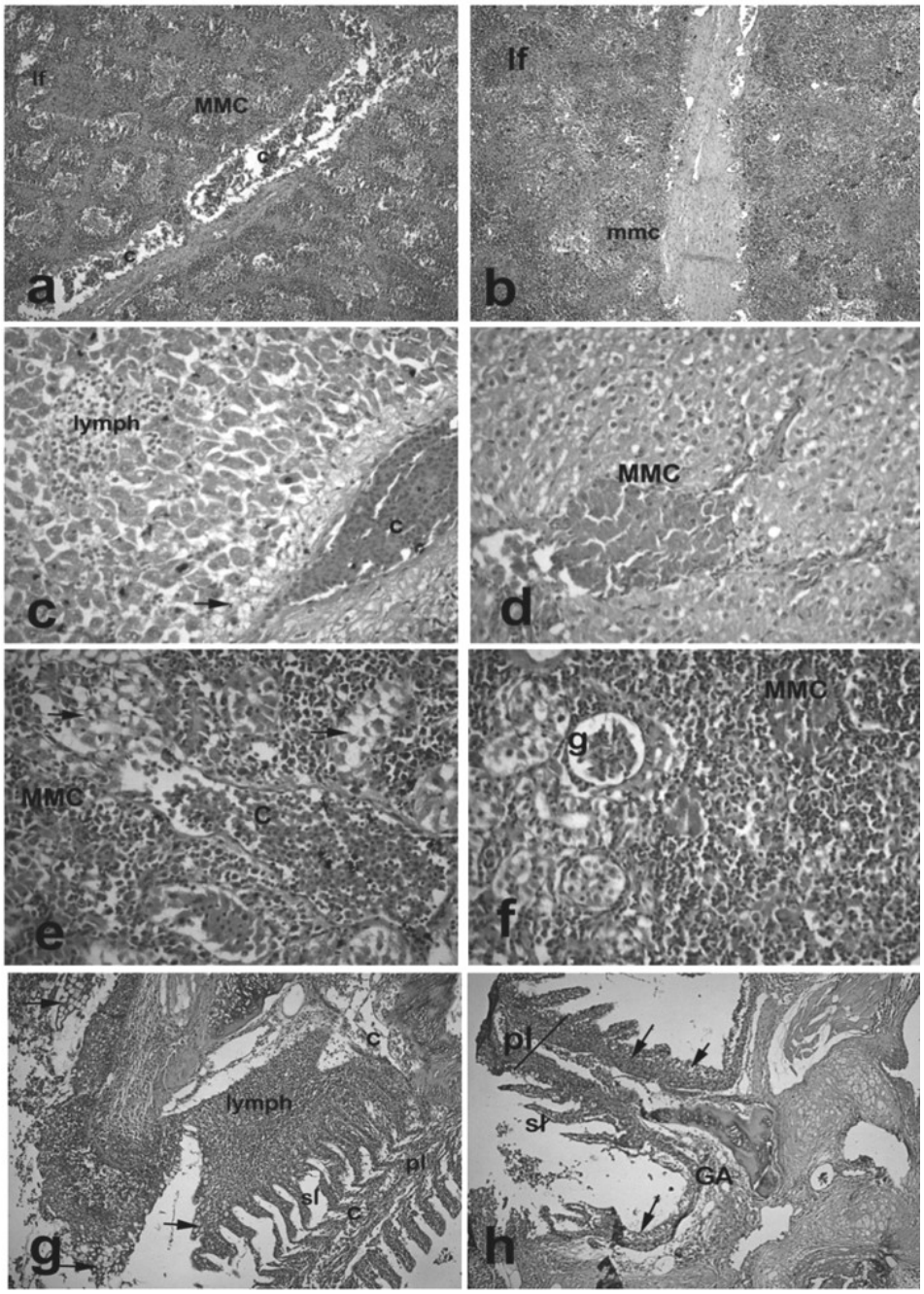
Group	Time	Leucocytic count $\times 10^3/\mu l$	Heterophils $\times 10^3/\mu l$	Lymphocytes $\times 10^3/\mu l$	Monocytes $\times 10^3/\mu l$	Eosinophils $\times 10^3/\mu l$	Basophils $\times 10^3/\mu l$
Control	1 week	25.49 ± 0.54^b	8.54 ± 0.22^b	15.17 ± 0.37^b	1.13 ± 0.05^a	0.49 ± 0.04^a	0.16 ± 0.004^a
	3weeks	26.31 ± 0.25^c	8.87 ± 0.19^c	15.34 ± 0.45^b	1.29 ± 0.03^b	0.65 ± 0.06^a	0.15 ± 0.01^a
	5 weeks	24.51 ± 0.16^d	7.79 ± 0.07^d	14.73 ± 0.11^c	1.22 ± 0.04^b	0.64 ± 0.01^a	0.12 ± 0.007^a
Infected	1 week	36.89 ± 0.39^a	11.54 ± 0.13^a	23.54 ± 0.41^a	1.14 ± 0.02^a	0.51 ± 0.05^a	0.15 ± 0.009^a
	3weeks	29.85 ± 0.27^b	9.93 ± 0.13^b	17.92 ± 0.46^a	1.19 ± 0.02^b	0.66 ± 0.07^a	0.14 ± 0.006^a
	5 weeks	28.34 ± 0.22^c	8.68 ± 0.09^c	17.67± 0.21^b	1.20 ± 0.08^b	0.65 ± 0.02^a	0.12 ± 0.002^a
β-glucan	1 week	26.17 ± 0.26^b	8.85 ± 0.21^b	15.69 ± 0.30^b	0.92 ± 0.26^a	0.55 ± 0.04^a	0.16 ± 0.01^a
	3 weeks	32.25 ± 0.26^a	10.68 ± 0.17^a	18.40 ± 0.35^a	2.38 ± 0.10^a	0.65 ± 0.08^a	0.14 ± 0.004^a
	5 weeks	39.87 ± 0.30^b	16.54 ± 0.42^b	20.16 ± 0.81^a	2.40 ± 0.09^a	0.65 ± 0.01^a	0.13 ± 0.006^a
β-glucan infected	1 week	26.22 ± 0.21^b	8.83 ± 0.17^b	15.59 ± 0.20^b	1.18 ± 0.04^a	0.47 ± 0.03^a	0.15 ± 0.009^a
	3 weeks	32.65 ± 0.31^a	10.84 ± 0.16^a	18.75 ± 0.46^a	2.26 ± 0.05^a	0.66± 0.09^a	0.14 ± 0.010^a
	5 weeks	43.34 ± 0.47^a	١٨.60 ± 0.25^a	٢١.67 ± 0.70^a	2.26 ± 0.07^a	0.68 ± 0.009^a	0.13 ± 0.008^a

Superscript with different letters in the same colum at the same week are significant at ($p < 0.05$)

Table 3: *Biochemical parameters (mean value ± SE) in different experimental groups*

Group	Time	AST U/L	ALT U/L	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl	Glucose mg/dl
Control	1 week	22.51 ± 0.51^{b}	17.57 ± 0.20^{b}	17.99 ± 0.38^{b}	0.38 ± 0.004^{b}	4.68 ± 0.20^{b}	54.23 ± 0.21^{b}
	3 weeks	23.98 ± 0.16^{b}	20.51 ± 0.29^{b}	19.78 ± 0.05^{b}	0.39 ± 0.008^{b}	4.66 ± 0.09^{b}	55.09 ± 0.50^{b}
	5 weeks	24.71± 0.29^{bc}	19.63 ±0.29^{bc}	18.69 ± 0.19^{b}	0.36 ± 0.008^{b}	4.88 ± 0.10^{b}	53.87 ± 0.39^{b}
Infected	1 week	32.59 ± 0.16^{a}	30.91 ± 0.10^{a}	33.26± 0.26^{a}	0.40 ± 0.005^{a}	8.69 ± 0.21^{a}	78.06 ± 0.30^{a}
	3 weeks	42.48 ± 0.14^{a}	40.09 ± 0.21^{a}	36.98± 0.24^{a}	0.42 ± 0.014^{a}	7.87 ± 0.16^{a}	70.06 ± 0.29^{a}
	5 weeks	43.68 ± 0.23^{a}	42.23 ± 0.22^{a}	28.40 ± 0.22^{a}	0.40 ± 0.004^{a}	6.79 ± 0.22^{a}	58.60 ± 2.4^{a}
β-glucan	1 week	22.33 ± 0.05^{b}	17.36 ± 0.21^{b}	18.10 ± 0.18^{b}	0.37 ± 0.006^{b}	4.35 ± 0.12^{b}	53.68 ± 0.31^{b}
	3 weeks	21.37 ± 0.53^{c}	18.69 ± 0.24^{c}	17.66 ± 0.30^{c}	0.37 ± 0.009^{b}	3.29 ± 0.15^{c}	54.24 ± 0.34^{b}
	5 weeks	23.61 ± 0.40^{c}	18.82 ± 0.31^{c}	15.68 ± 0.41^{c}	0.34 ± 0.006^{b}	2.49 ± 0.25^{c}	51.30 ± 0.56^{b}
β-glucan infectd	1 week	23.01 ± 0.07^{b}	17.21± 0.24^{b}	17.70 ± 0.09^{b}	0.38 ± 0.003^{b}	4.39 ± 0.09^{b}	53.87 ± 0.25^{b}
	3 weeks	21.54 ± 0.51^{c}	18.92± 0.06^{c}	17.23 ± 0.19^{c}	0.38 ± 0.003^{b}	3.44 ± 0.12^{c}	54.32 ± 0.30^{b}
	5 weeks	23.91 ±0.52^{bc}	19.10± 0.24^{bc}	16.18 ± 0.39^{c}	0.35 ± 0.007^{b}	2.92 ± 0.21^{c}	52.18 ± 0.18^{b}

Superscript with different letters in the same colum at the same week are significant at ($p < 0.05$)



Figures (a), (c), (e) and (g) showing spleen, liver, kidney and gills of the infected catfish (*Clarias gariepinus*) group respectively. Photos (b), (d), (f) and (h) showing spleen, liver, kidney and gills of the infected β-glucan catfish (*Clarias gariepinus*) group respectively.

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الملخص العربي

دراسات باثولوجية اكلينيكية على دم اسماك القط الافريقي المصابة بمرض التسمم الدموي السودوموناس

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تم عمل هذه الدراسة بغرض التعرف على تأثير الإصابة بمرض التسمم الدموي السيدوموناسي على دم أسماك القرموط الأفريقي ودراسة تأثير الوقاية باستخدام البيتاجلوكان كمحفز مناعي طبيعي. وقد أستخدم في هذه الدراسة ٢٤٠ سمكة وتم تقسيمها إلى أربع مجموعات (المجموعة الضابطة ، المجموعة المصابة بميكروب السيدوموناس فلورسنس، مجموعة البيتاجلوكان ومجموعة البيتاجلوكان المصابة). و قد تم تجميع عينات الدم من المجموعات المختلفة فى الاسبوع الاول والاسبوع الثالث والاسبوع الخامس لفصل البلازما وفصل المصل بهدف اجراء الاختبارات الدموية والكيميائية. أوضحت النتائج وجود انخفاض معنوي في عدد خلايا الدم الحمراء، نسبة الهيموجلوبين ونسبة الخلايا المثقلة وأيضا زيادة في عدد خلايا الدم البيضاء خاصة المتعادلة والخلايا الليمفاوية بالاضافة الى زيادة كل من إنزيمات الكبد والكلى والجلوكوز في الأسماك المصابة بالمقارنة بالمجموعة الضابطة.. أما أسماك مجموعتى البيتاجلوكان والبيتاجلوكان المصابة فقد أظهرت زيادة معنوية في عدد خلايا الدم الحمراء، ونسبة الهيموجلوبين ونسبة الخلايا المضغوطة وزيادة في عدد خلايا الدم البيضاء خاصة المتعادلة والخلايا الليمفاوية والاحادية بالاضافة الى وجود انخفاض غير ملحوظ لكل من إنزيمات الكبد والجلوكوز مع انخفاض ملحوظ في وظائف الكلى مع تقدم التجربة بالمقارنة بالمجموعة الضابطة. وكذلك لوحظ انخفاض فى نسب النفوق بالأسماك المغذاة على العليقة المضاف إليها البيتاجلوكان. كما اسفرت النتائج النسجومرضية للأنسجة عن وجود اضطرابات دورية مع تغيرات هدامة والتهابات في معظم الأعضاء المختبرة للأسماك المصابة، بالاضافة الى زيادة فى نشاط الميلانومكروفاج الموجودة فى الكبد والطحال والكلى بالأسماك المغذاة على العليقة المضاف إليها البيتاجلوكان مقارنة بالمجموعة الضابطة. كما اوصت النتائج باستخدام البيتاجلوكان كمحفز مناعي طبيعي في عليقة أسماك القط الافريقي.