

EFFECT OF ADDING GARLIC *ALLIUM SATIVUM* POWDER IN DIET ON
HEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL CRITERIA OF
COMMON CARP *CYPRINUS CARPIO* L.

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ABSTRACT

Common carp was fed at a rate of 9 ± 0.4 g/fish for a period of 56 days on four experimental diets with three replications per diet, including the addition of garlic powder to a diet containing 0 % (control diet, T1), while T2, T3 and T4 at 1.00, 1.50 and 1.75 %, respectively. Fish fed on experimental garlic-containing diet show a significantly changes ($P \leq 0.05$) in Packed Cell Volume and Hemoglobin as compared with the control diet. There were no significant differences in MCHC and neutrophil counted, while the albumin value of fish fed on the 2nd diet increased significantly ($P \leq 0.05$) among all treatments. The values of ALT (except for diet 2) and AST decreased significantly ($P \leq 0.05$) compared with control diet. Results of histological examination of intestine for fish fed on garlic at 1.00% showed presence of edema at the top of the villi, while the lesion was more severe in intestine of fish fed on garlic at 1.5 and 1.75%, Likewise, histological examination of the liver of carp fed garlic at 1.00, 1.5 and 1.75% showed histopathological alteration - represented by pyknosis and vacuolar degeneration of hepatocyte with atrophy of pancreas and hemorrhage. It is evident from the above that the addition of garlic powder has led to an improvement in blood and biochemical characteristics, but the addition of garlic has had some negative effects on the histological characteristics of the intestine and liver, possibly as a result of the long-term feeding of fish to these proportions of garlic.

Keywords: medical plant blood picture , total protein, globulin.

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تأثير اضافة مسحوق الثوم *Allium sativum* على المعايير الدموية و الكيموحياتية والنسجية

لأسماك الكارب الشائع *Cyprinus carpio* L.

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المستخلص

تم تغذية اسماك الكارب الشائع بمعدل 9 ± 0.4 غم / سمكة لمدة 56 يوماً على أربع علائق تجريبية وبقوات ثلاث مكررات لكل عليقة . تم إضافة مسحوق الثوم إلى العلائق بنسبة 0% (عليقة ضابطة، المعاملة الاولى)، و 1,00 و 1.50 و 1.75% إلى المعاملات الثانية والثالثة والرابعة على التوالي. بينما ارتفعت قيم PCV و Hb للأسماك التي تغذيتها على الثوم معنوياً ($P \leq 0.05$) عن عليقة السيطرة. بينما قيم الالبومين ارتفعت معنوياً للأسماك المغذاة على المعاملة الثانية عن كافة العلائق التجريبية. بينما انخفضت قيم ALT و AST (باستثناء المعاملة الثانية) معنوياً مقارنة بالعليقة الضابطة. أظهرت نتائج الفحص النسيجي لأمعاء الأسماك التي تتغذى على العليقة الحاوية على الثوم بنسبة 1.00% وجود الخرب في قمم الزغابات ، بينما كانت الآفة أكثر شدة في أمعاء الأسماك التي تم تغذيتها على الثوم بنسبة 1,5 و 1,75%. وبالمثل ، أظهر الفحص النسيجي لكبد الكارب الذي تغذى على الثوم بنسبة 1,00 و 1,5 و 1,75% حدوث تغيرات تغييرات مرضية نسجية تمثلت بتغلظ والتكس الفجوي للخلايا الكبدية والكيموحيوية، ولكن إضافة الثوم كان له بعض الآثار السلبية على الخصائص النسجية للأمعاء والكبد، ربما نتيجة المدة الزمنية لتغذية الأسماك على الثوم وبنسب مختلفة.

الكلمات المفتاحية: النباتات الطبية، البروتين الكلي، الكلويولين، الصورة الدموية، تقطيع نسيجي

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INTRODUCTION

Intensive fish farming is an effective way to increase fish production, but this may lead to an increase in infection and the spread of various diseases due to the stress (1, 15) caused by the decrease in the concentration of dissolved oxygen, high density and circulation, which causes the overuse of antibiotics accumulate in the various fish tissues, may pose numerous risks to human health as well as to human health as they move through the food chain (7). In general, plants have a variety of functions due to the presence of various active compounds such as alkaloids, flavonoids, pigments, phenols, terpenoids, steroids and essential oils (9). Medicinal plants have contributed to the promotion of various activities such as stress control, (24) growth enhancement, increased feed intake and enhanced immunity in aquaculture-related activities (30) by increasing insulin levels or

feed fish on new sources of high nutritional value (23). The use of garlic stimulated growth in common carp *Cyprinus carpio* L. (22). A number of studies have examined the effect of garlic addition on growth criteria and hematological parameters of *Clarius gariepinus* (17). The aim of this study is to study the effect of adding different levels of garlic powder on hematological, biochemical parameters and Histopathological changes of common carp *Cyprinus carpio* L.

MAERIALS AND METHODS

Experimental diets: Common carp were fed on four experimental balanced diets in terms of their content of crude protein (25.45%) and metabolic energy (13.17ME\Kg). Fish fed at diets contained dry garlic powder *Allium sativum* at 0, 1.00, 1.50 and 1.75% for T1 (control diet), T2, T3 and T4 respectively (Table 1).. Fish were fed at 3% of the wet body live weight twice a day.

Table 1. Dietary ingredients and chemical composition (%DM) of the experimental diets containing different percentages of Garlic powder

Diets		Control 0%	Garlic 1.25%	Garlic 1.5%	Garlic 1.75% (T4)
Ingredients		(T1)	(T2)	(T3)	
Garlic powder		-	1.00	1.5	1.75
Animal protein		10.00	10.00	10.00	10.00
Soybean meal		30.00	30.00	30.00	30.00
Local barley		20.00	20.00	20.00	20.00
Yellow corn		18.50	18.50	18.50	18.50
Wheat bran		19.00	19.00	19.00	19.00
Food salt		1.00	1.00	1.00	1.00
Vita. & Miner. Mix.		0.50	0.50	0.50	0.50
Lime stone		0.50	0.50	0.50	0.50
binder (Bentonite)		0.50	0.50	0.50	0.50
Chemical composition					
Crude protein	Ether extract	Ash	Crude fiber	Nitrogen free extract	ME (MJ/Kg)
25.45	3.54	6.97	4.75	52.2	13.17

Fish were acclimatized for two weeks with a glass aquarium environment (72 L), containing 10 fish per each aquaria. After the acclimatization had ended, the fish has been fed on experimental diets for 56 days. Fish was randomly distributed at a weight rate of 9 ± 0.4 g / fish on experimental diets. Water temperature water ranged between 23 -26°C is controlled by air conditioners, pH is ranged 7.3-7.6 and oxygen dissolved is ranged between 6.6-7.8 mg / L.

Collection of blood samples

Blood samples were collected from the caudal vein by means of a 2.5 ml plastic syringe and divided into two parts..

The first section of the blood was placed in an anticoagulant-free glass tube for serum isolation (10), the serum were kept -20°C till the determination of biochemical tests. Serum total protein, albumin, globulin, glucose, triglycerides concentrations, as well as the activity of AST and ALT enzymes. The second parts of blood samples were kept in EDTA tubes, and used for determination of blood picture (Packed Cell Volume, Hb, MCHC, neutrophil and lymphocytes(%)).The tubes were placed diagonally at room temperature for 30 minutes and then placed in a centrifuge (3000 cycles for 10 minutes) to obtain the serum that was placed in clean

plastic tubes and kept at -20°C until the tests for biochemical analysis were carried out

Blood smears: The packed cell volume (PCV) was calculated using the method provided by (10), and the hemoglobin (Hb) and mean corpuscular hemoglobin (MCHC) concentrations were calculated using the method described by (11). The values of neutrophils and lymphocytes were estimated based on (25). Total serum protein, albumin and globulin concentrations estimated by (16 and 20). Based on a kit provided by the International Medical Reagents Company, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), glucose, and triglyceride levels were determined.

Histopathological test: In order to perform the anatomical characteristics of the fish, the method used to damage the spinal cord was used to anesthetize the fish (19) if a longitudinal incision was made from the outlet opening to the gills cap, then a transverse incision was made towards the lateral line. Tissue samples were collected from the liver and intestine and then preserved in buffer formalin 10% until the slides were prepared for histological examination (19). The internal organs of the fish, represented by the intestine and liver, were extracted, they were preserved in a buffer solution at a concentration of 10% for the purpose of histological examination. Tissue slices with a thickness of 5 microns were prepared using a microtome, then the slides were colored with Harris hematoxylin and eosin alcoholic stain (20) and then the slides were examined by light microscopy for the samples studied.

Analytical statistics: The Statistical Package for Social Science (SPSS, Version 25) used the complete randomized design in data analysis to examine the effect of experimental treatments on the studied criteria and the significant differences between the mean value examined by Duncan multiple range test.

RESULTS AND DISCUSSION

Blood picture results: Packed cells volume and hemoglobin content: The results of the statistical analysis showed that there were a significant differences ($P \leq 0.05$) between fish obtained different levels of garlic in PCV criteria. Garlic powder was added to the experimental diets (the second, third, and

fourth treatments at three levels, resulting in significant ($P \leq 0.05$) superiority over the control diet (the first treatment) of 23.33, 24.00, 26.33, and 16.67%, respectively (Table 2). The results of the statistical analysis of hemoglobin showed that the hemoglobin values of fish fed on garlic-containing diets reached 8.30, 7.27, and 8.57 g/100 ml, for the 2nd, 3rd and 4th diets respectively, which increased significantly ($P \leq 0.05$) compared with control diet (T1) (5.93 g/100 ml). Table (2) showed that there were no significant differences in the mean corpuscular hemoglobin concentration (MCHC) due to the addition of garlic powder, as the values of this parameter were 32.93, 33.16, 33.93 and 33.07 (g/100 ml) for fish fed on diets 1st, 2nd, 3rd and 4th respectively. This significant improving effect of garlic on blood picture parameters may be related to its high content of reported that garlic was rich in vitamins necessary for blood building as thiamine, riboflavin and folic acid, also many amino acids that required for Hb building (18). These results are consistent with (27) of sterlet sturgeon *Clarias batrachus* which feeding on garlic-containing diets has led to a significant increase in the levels of PCV, hemoglobin.

Neutrophils and lymphocytes: In our innate immune system, neutrophils are white blood cells that play some very important roles. By producing antibodies, which are chemicals that help your body stop pathogens such as bacteria, viruses, fungi, parasites, and toxic chemicals, the role of lymphocytes is to fight infection. The addition of garlic powder had no significant effect on the number of neutrophils in different proportions, since the rate of this criterion was 18.00 (control diet), 17.45, 18.33 and 16.33 for fish fed on the second, third and fourth diets, respectively (Table 2). The statistical analysis results indicated that there were significant differences ($P \leq 0.05$) in the number of lymphocytes that decreased to 63.67, 75.00 and 64.67 (second, third and fourth diets) compared to control diets when garlic powder was added (88.33). These findings are not consistent with (27), which reported the highest number compared to control of lymphocytes obtained from garlic extract.

Table 2. Effect of Garlic powder supplementation on blood picture in common carp (Means \pm SE).

Diets	Criteria	PCV %	Hb g /100 ml	MCHC g /100 ml	Neutrophil %	Lymphocyte %
Control		16.67 \pm 0.33	5.93 \pm 0.33	32.93 \pm 0.33	18.00 \pm 3.92	88.33 \pm 2.33
1% Garlic		23.33 \pm 0.88	8.3 \pm 0.68	33.16 \pm 0.03	17.45 \pm 2.33	63.67 \pm 2.73
Garlic 1.50%		24.00 \pm 0.58	7.27 \pm 0.24	33.93 \pm 0.12	18.33 \pm 0.88	75.00 \pm 1.53
1.75% Garlic		26.33 \pm 0.88	8.57 \pm 0.15	33.07 \pm 0.13	16.33 \pm 0.33	64.67 \pm 2.40

*Means not sharing a common superscript letter are significantly differences ($P \leq 0.05$).

Effect of treatments on biochemical parameters: Statistical blood glucose analysis revealed that adding garlic powder to fish diets at varied doses resulted in a significant decrease ($P \leq 0.05$) in blood glucose levels of 47.33, 50.67, and 46.00 mg/100 mL, respectively, as compared to control diets (94.67) (Table 3). Although the criteria for cholesterol and triglycerides were not significantly different, with the exception of 1.75% of garlic (fourth diet) compared to the control diet, where the cholesterol values were 115.33 and 139.67 and the triglycerides were

192.67 and 276.33 mg/100 ml for fish fed on the fourth diet and control diet, respectively. The reduction in glucose levels may be due to that garlic may reduce glucose absorption from intestine in the same time garlic had no effects on food consumption as reported by (23). Rodge *et al.* (27) found a significant decrease in plasma glucose and triglycerides with increased levels of garlic in *Clarias batrachus*, S-allylcysteine sulfoxide, one of the components of garlic, is responsible for reducing the efficacy of blood sugar (29).

Table 3. Effect of garlic powder supplementation on glucose, cholesterol, and triglyceride in common carp (Means \pm SE).

Diets	Criteria	Glucose (mg\100 ml)	Cholesterol (mg\100 ml)	Triglyceride (mg\100 ml)
Control		*94.67 \pm 14.67	139.67 \pm 5.78	276.33 \pm 12.91
Garlic 1.00%		47.33 \pm 4.10	133.33 \pm 0.88	223.67 \pm 19.41
Garlic 1.50%		50.67 \pm 7.69	134.00 \pm 11.67	224.33 \pm 15.90
Garlic 1.75		46.00 \pm 1.73	115.33 \pm 3.92	192.67 \pm 18.68

*Means not sharing a common superscript letter are significantly differences ($P > 0.05$).

Total protein , albumin and globulin

Total protein values were significantly higher ($P \leq 0.05$) than the control diet, with approximately 34.00, 34.04, 34.67 and 28.00 gm/100 ml for 2nd, 3rd, 4th and 1st diets respectively (Table 3). This may be due to the fact that garlic may enhance the absorption of free amino acids by white muscles, which in turn enhance protein synthesis (14). The results of the statistical analysis showed that there was a significant increase ($P \leq 0.05$) in the of albumin reached 15.33g\100ml for fish fed on the fourth diet (1.75% garlic) to 15.33 g/100 ml (Table 3) compared to control diet (13.33 gm / 100 ml). No significant differences were recorded for fish fed on the second and third diets compared with control. However, the addition of garlic at three levels showed a significant increase ($P \leq 0.05$) in

globulin compared to control diets of 22.33, 22.34, 19.33 and 15.67 g/100 ml for 2nd , 3rd, 4th and 1st diets, respectively. Because albumin, globulin, and total serum proteins have a wide range of vital functions in fish. These results are consistent with the findings of many researchers that the addition of garlic significantly increases some of the blood parameters, including a significant increase in total protein, albumin and globulin of common carp (8). The positive results accompanying the addition of garlic powder to the above criteria also included the ALT criterion (IU/L) which significantly decreased ($P \leq 0.05$) when adding garlic powder was added by 1.50% and 1.75% which was 274.33 and 199.67, respectively. Although no significant differences were reported for the ALT characteristic of fish fed on control and second

diets (1.0% garlic), which amounted to 193.35 and 253.67, respectively. The positive effect of the addition of garlic powder appeared more clearly with regard to the results of the AST analysis, as all parameter values decreased

significantly ($P \leq 0.05$) compared to the control diet, at 250.67, 249.67 and 244.67 (IU/L) for the second, third and fourth diets, respectively, and at 297.45(IU/L) (diet1).

Table 4. Effect of garlic powder supplementation on total protein, albumin, globulin, ALT and AST in common carp (Means \pm SE).

Diets	Criteria	Total protein g\100ml	Albumin g\100ml	Globulin g\100ml	ALT IU\L	AST IU\L
Control		28.00 \pm 1.12 b	13.00 \pm 0.58 b	15.67 \pm 1.86 b	*253.67 \pm 15.06 a	279.35 \pm 5.78 a
Garlic 1.00%		34.00 \pm 1.15 a	11.67 \pm 0.67 b	22.33 \pm 0.57 a	274.33 \pm 3.84 a	250.67 \pm 5.17 b
Garlic 1.50%		34.04 \pm 1.15 a	11.67 \pm 0.67 b	22.34 \pm 0.67 a	193.35 \pm 17.33 b	249.67 \pm 4.67 b
Garlic 1.75%		34.67 \pm 0.67 a	15.33 \pm 0.88 a	19.33 \pm 0.33 a	199.67 \pm 17.33 b	244.67 \pm 2.60 b

*Means not sharing a common superscript letter are significantly differences ($P > 0.05$).

It is beneficial to focus attention on changes in the activity of AST and ALT as it improves the synthesis of amino acids and changes in the activity of aminotransferase in the liver (2). *Allium sativum* may alter the activity of AST and ALT by a variety of chemical, biological and physiological factors, or by interrupting the Krebs cycle, decreases the activity of the Krebs cycle by decreasing the activity of α -ketoglutarate mediators, *Allium sativum* may stabilize the cell membrane and protect the liver from harmful agents and toxic substances with free-radical damage to liver cells that are reflective of the liver. Garlic helps the liver to maintain its normal function by accelerating the ability of its cells to regenerate. These results are consistent with the findings of (21) in Nile tilapia.

Histopathological

Examination: The results of the histological examination of intestinal fish fed at the 1.00% garlic (second diet) show normal structure as in fig 1.,while there was edema at the top of the villi (figure 2), the lesion in intestinal fish treated with 1.5 and 1.75 percent was more severe (Figures 3, 4, 5). Adibmoradi *et al.* (3) discovered histological and morphological changes in the intestine of fowl when 2% garlic was introduced. Although Agbebi *et al.* (4) indicated that there were no histological changes in African catfish *Clarias gariepinus* fed garlic-containing. It should be noted that limited scientific literature on the effect of garlic supplementation on histological examination of fish has been found. Ezzat (13) found that the addition of fenugreek oil improved growth performance and histological properties.



Figure 1. Showed the intestine in fish fed diet with garlic at rate 1.00% showed normal structure muscular layer (ML)yellow star (*), epithelial enterocyte (EE) blue row, goblet cells (GC)red row with edema at the apex of villi (black row)i, H&E 10 * 2.3X

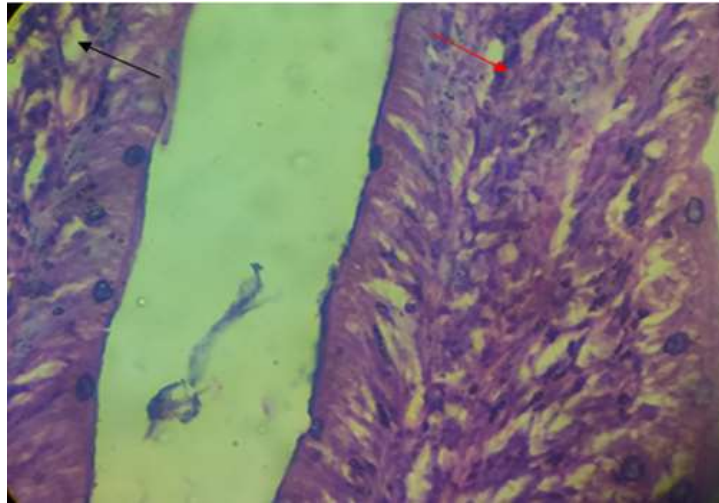


Figure 2. Showed the intestine in fish fed diet with garlic at rate 1.50% showed edema (black row), infiltration of inflammatory cells red row, H&E, 40 * 1X

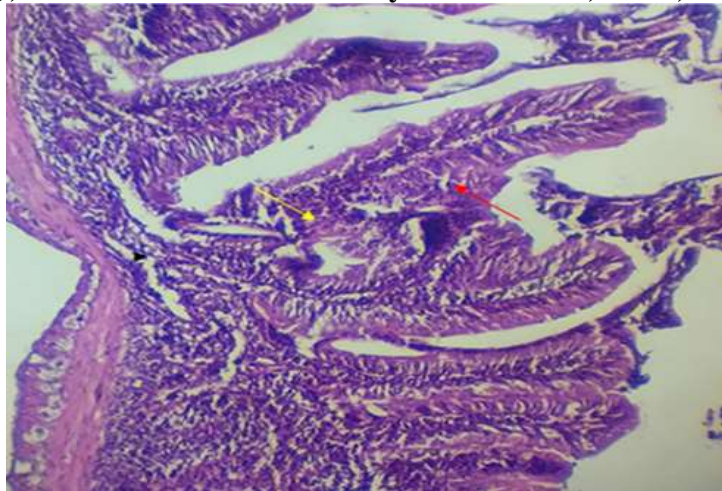


Figure 3. Showed the intestine in fish fed diet with garlic at rate 1.5% showed vacuolar degeneration (black row) between ML and serosa (short black row), adhesion between the villi (red row) and infiltration of inflammatory cells (yellow row), H&E, 10*1X

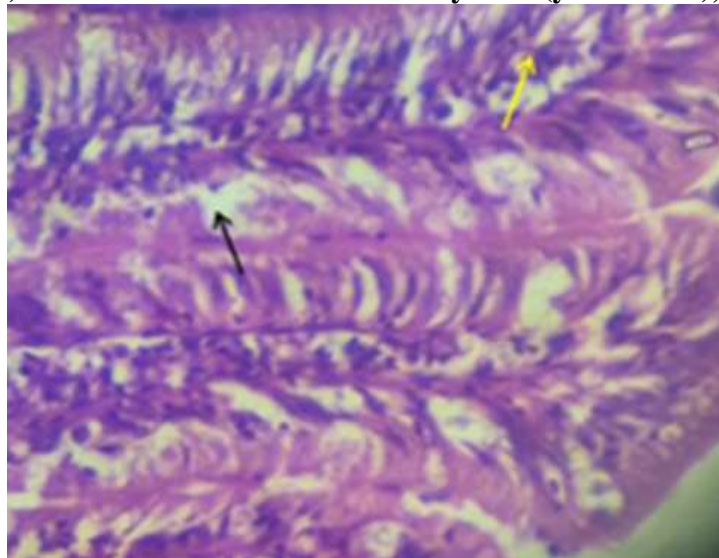


Figure 4. Showed the intestine in fish fed diet with garlic at rate 1.5% showed vacuolar degeneration (black row) and infiltration of inflammatory cells (yellow row), H&E,40*1X

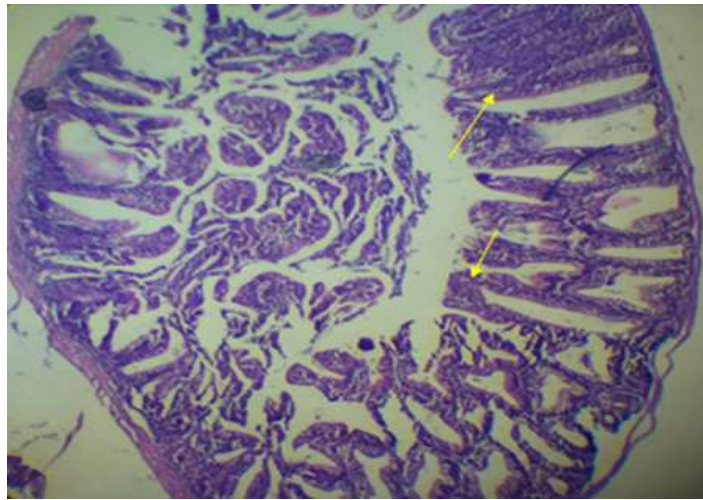


Figure 5. Showed the intestine in fish fed diet with garlic at rate 1.75% severe adhesion between villi (yellow row), H&E,4*1X

Liver is one of the most important organs because of its detoxification capacity and its vital metabolic functions. Psychotic hepatocyte nucleation and vacuolar degeneration with pancreatic tissue atrophy and bleeding have been reported to thicken at rates of 1.0 percent, 1.5 percent, and 1.75 percent for 56 days of carp fish fed garlic-containing diets. (Figures 6, 7 and 8). Al-Salahy and Mahmoud (5) reported that Histopathological changes had occurred in the liver of African fish *Chrysichthys auratus* had been treated with garlic (dose 2 g / kg) for five days, when fed on a diet, containing 2.8% garlic. The severity of lesion are more clearly in the liver of fish fed on high percent garlic 1.75% containing diet which represented by necrotic pancreatic tissue, edema with infiltration of inflammatory cells, wall thickening of arterioles with severe vacuolar degeneration and dilatation of sinusoids as in

figure 9 and10. In our current research, changes in the intestine and liver of garlic-treated carp may be associated with the body's natural defense reaction or adaptation to garlic as an external substance, or may result from the negative effect of garlic as a result of its long-term use as demonstrated by (4). The significance of garlic, its properties for controlling diseases and its limited side effects. Notwithstanding the known benefits of the active substance allicin in garlic, it may have a harmful effect on the intestines of fish that is degraded to dialysulphate (24) and may interfere with intestinal metabolic processes (11). Thus, high garlic concentrations over a long period of time can lead, depending on the type of fish, to imbalances in the ion balance, cell damage and differences in the vitality of the intestinal cell membrane, liver, and blood vessels.

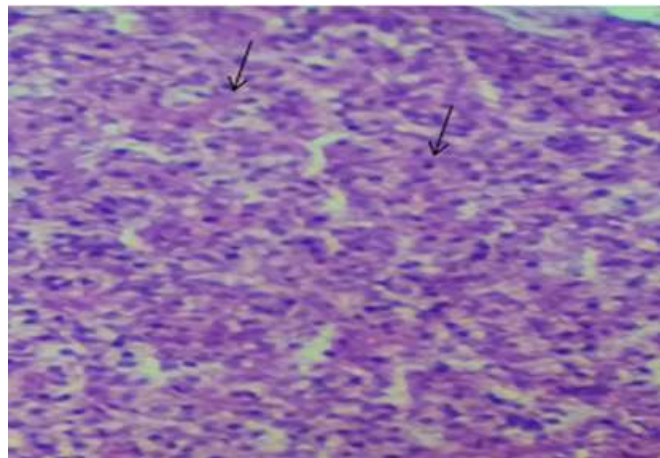


Figure 6. Showed the liver in fish fed diet with garlic at concentration 1.25% show normal structure with nuclear pyknosis (black row), H&E,10*4X

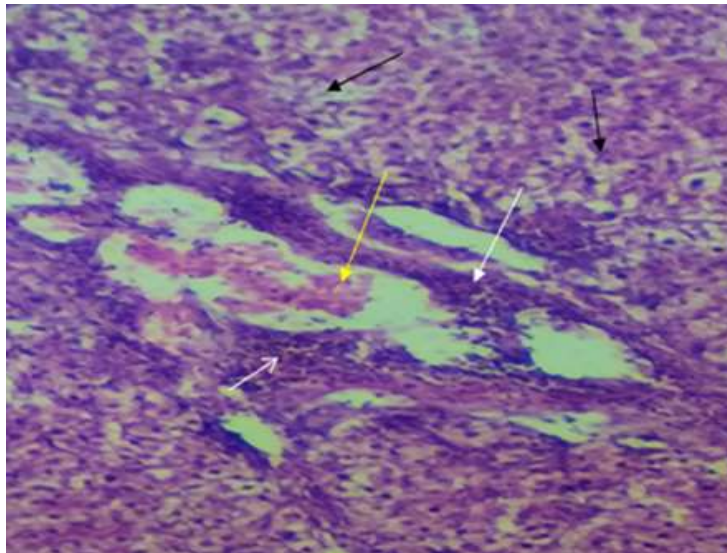


Figure 7. Showed the liver in fish fed diet with garlic at concentration 1.5% show vacuolar degeneration (black row), congestion (yellow row) with perivascular cuffing (white row) H&E,10*1X

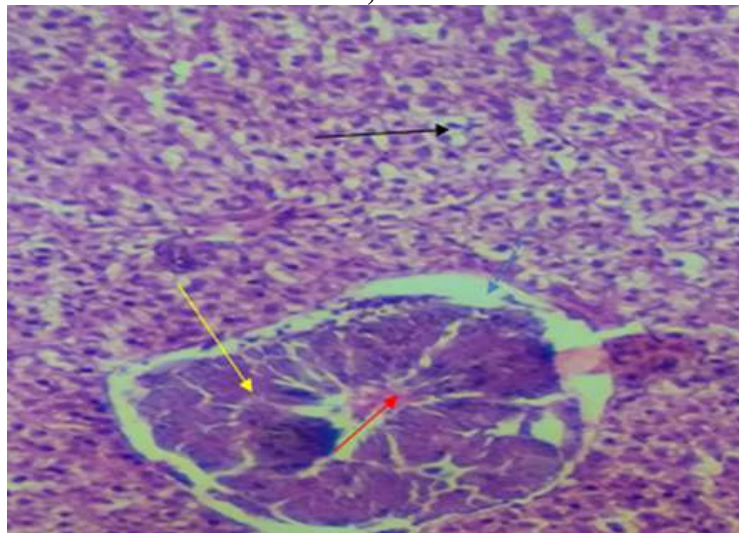


Figure 8. Showed the liver in fish fed diet with garlic at concentration 1.75% show vacuolar degeneration (black row), atrophy of pancreatic tissue (yellow row) with hemorrhage (red row) and edema (blue row) H&E,10*1X

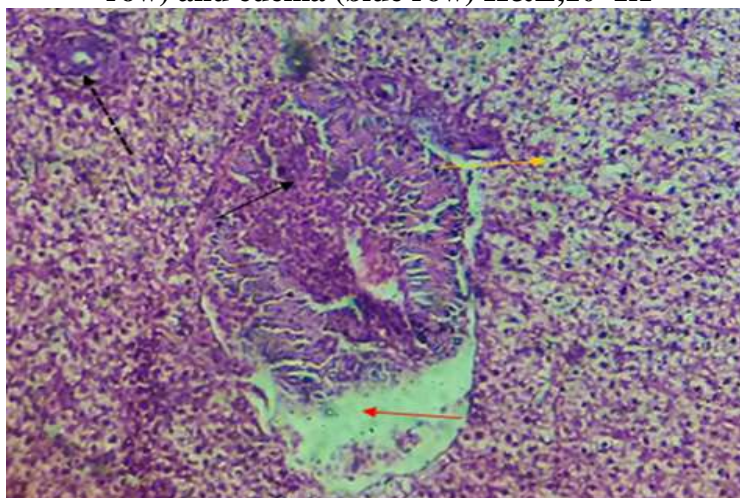


Figure 9. Showed the liver in fish fed diet with garlic at concentration 1.75% show necrotic pancreatic tissue (black row) edema (red row) infiltration of inflammatory cells (yellow row), thickening of bile ducts wall (black dot row) H&E,10*1.8X

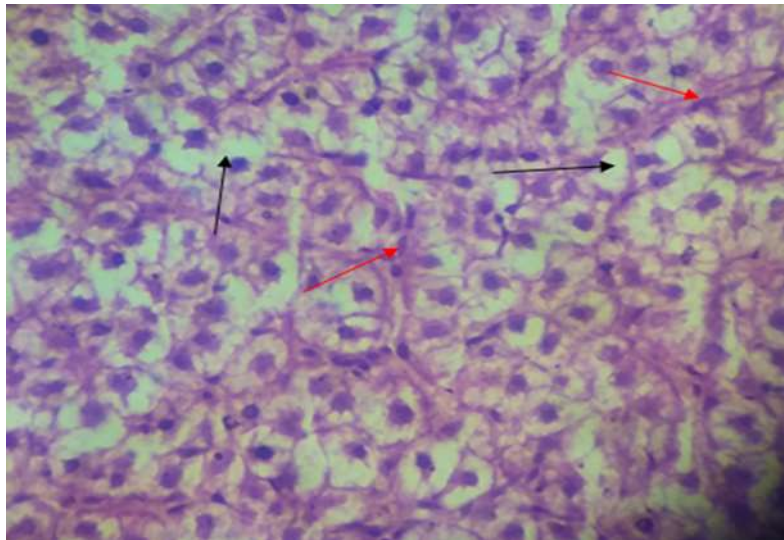


Figure 10. Showed the liver in fish fed diet with garlic at rate 1.75% show severe vacuolar degeneration (black row), dilatation of sinusoids (red row) H&E,40*1X

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