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Effect addition of *Cinnamomum cassia* on treatment of pathological infections in *Cyprinus carpio* L. fingerlings

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Abstract

The goal of this study was to isolate and identify the causative agents that causes death in Yankee Hatch / Erbil fingerling *Cyprinus carpio* and to investigate the effect of Cinnamon on the infected fish handled. Both *Staphylococcus aureus* and *Escherichia coli* are strongly isolate followed by *Aeromonas hydrophila* and *Klebsiella pneumonia* were isolated from liver, kidney and intestine which cause histopathological changes in these organs, characterized by fibrosis in liver, coagulative necrosis in renal tubules in the kidney and sever enteritis. *Cinnamomum cassia* added to the ration of infected fish at concentration 0.75, 1 and 1.5% for eight weeks. The histopathological examination reveals that the 1.5% is best the percentage used as food additive for repair and regenerative tissue damage in the liver, kidney and intestine. These study conclude that *C. cassia* have been used as additive food in fish feed ration at 1.5% and have important role in regenerative tissue damage and keep fish in health status.

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Introduction

One of the fastest growing industries in the animal products industry is aquaculture, it is a good source of protein for human consumption as percentage 77% of fish directly and 23% indirect consumption (1). Freshwater fish Cypinidies are the important productive fish in the world and are an important protein source for human, but it susceptible to disease due to stressful condition as overcrowding of breeding, bad management, feeding, physiological status of fish and suppression of immune system. all these condition lead to loss defense mechanisms and facilitates the spread of pathogenic microorganisms which may inhabiting in the water environment and cause disease and high mortality (2,3). Antibiotic and chemotherapeutic agents are more common used in aquaculture for controlling fish diseases and may be used for long period these lead to high resistance of microorganisms, so it necessary to elevate fish defense mechanisms through administration of immune stimulus as *Spirulina spp*. which consider a good promotion growth and production in *C. carpio* (4) and some additive plant act as scavenger agent for removal mycotoxin (5). Other act as alternative for antibiotic in fish farming (6-9). Administration of medicinal plants is favorite method can be treat bacterial diseases as gram positive and gram negative bacteria as *Staphylococcus spp*., *Streptococcus* and *Aeromonas hydrophila* (10,11) one of these plant is *Cinnamomum cassia* has biological properties as analgesic carminative, hemostatic, antiseptic, antispasmodic, insecticidal, antiparasite and antifungal (12). Cinnamon stimulate innate immunity, so it used to treat disease, inflammation gastrointestinal disorder and urinary infections (13,14). The chemical composition which consist from flavonoids, saponins and tannins have antioxidant, anti-inflammatory activity and have ability to reduce effects of free radicals to keep cell physiology (15). The aims of the study are isolation and identification the causative agents that cause death in illness fish in Yankee hatch and to determine therapeutic effects of food supplementation of *Cinnamomum cassia* for treated illness fish.

Materials and methods

During period January - May 2020, one hundred fifty illness fingerlings (*Cyprinus carpio*) (16) 15± 2 gm were brought from yankee hatch /Erbil to fish laboratory /agriculture college in Mosul university, fish keep in aquarium 40*60*40cm with aerated contentious drainage water and temperature 18-20ºC. Fish are divided in to the first group have thirty fish were pithing and organs liver, kidney and intestine were collocated (17) and divided in to two subgroups: first sub group was preserved in broth for bacterial isolation until reach to the lab. other parts of internal organs were taken and kept in brain heart infusion broth (18) then incubated at 37ºC for 24hr (2). All broths were cultured on blood agar, MacConkey agar, EMB agar and Mannitol Salt Agar. The isolates were grown on agar plates for morphological, biochemical tests and VITEK® 2 compact for final identification of isolated bacteria (19,20). Whilst second sub group was fixed in 10% formalin for histological examination. The samples were dehydrated by ascending ethanol and clearance with xylene, then harding with paraffin 50˚C and scation with 5micron and staining with hematoxylin and eosin for histological examination (21). Second group included one hundred twenty fish were feeding on commercial pellet and divided randomly to four sub group one of them is consider control group were fish feeding only commercial pellet (Table 1) while the other three groups fish were feeding with commercial pellets with rode powder of *Cinnamomum cassia* at percentage 0.75, 1 and 1.5% for 8 weeks (22) after that organs liver, kidney and intestine were collected for histopathological examination.

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

Ingredients	Control	Cinnamon 0.75%	Cinnamon 1.0%	Cinnamon 1.5%
Cinnamon powder	-	0.75	1	1.5
Animal protein	10	10	10	10
Soybean meal	30	30	30	30
Local barley	20	20	20	20
Yellow corn	18.5	18.5	18.5	18.5
Wheat bran	19	19	19	19
Food salt	1	1	1	1
Vita. & Miner. Mix.	0.5	0.5	0.5	0.5
Lime stone	0.5	0.5	0.5	0.5
Binder (Pentonite)	0.5	0.5	0.5	0.5
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

*Calculated according to (23) equation: ME (MJ/Kg) = Protein X 18,8 + Fat X 33,5 + NFE X13,8 (11).

Results

The results of culture showed isolation of 4 types of pathogenic bacteria from infected fishes that included 24 bacterial isolates which high percentage from both *S. aureus and E. coli*, followed by *A. hydrophila*, and finally *K. pneumonia* as in (Table 2).

Histopathological examination of the liver, kidney and intestine organs in the fish infected with the bacterial *Aeromonas* and *Staphylococcus* reveals sever pathological lesions characterized by fibrosis, multifocal infiltration of inflammatory cells with hemorrhage and vacuolar degeneration in hepatocyte (Figure 1), while microscopic analysis of kidney reveals coagulative necrosis, interstitial nephritis with hemorrhage (Figure 2), there was also sever enteritis in an infected fish with *Staphylococcus* characterized by necrosis of the muscular layer with adhesion of the villi and loss the crypt with necrosis (Figure 3).

Histological analysis of the organs of a diseased fish treated with various concentration of *C. cassia* 0.75, 1 and 1.5% indicates various healing stages in the liver, kidney and intestine organs. Microscopic examination of liver of fish in group treated with 0.5% of the *C. cassia* exhibit vacuolar degeneration, infiltration of inflammatory cells with hemorrhage and fibrosis and edema (Figure 4) whereas vacuolar degeneration and dilatation of the sinusoid investigated in the liver of fish in that group treated with *C. cassia* at 1% (Figure 5), while in comparison to the microscopical examination of the liver in the fish was treated with 1.5%, the liver appear normal there was only moderate infiltration of inflammatory cells (Figure 6).

While microscopic examination of kidney in fish treated with 0.75 and 1% of *C. cassia* exhibit coagulative necrosis in renal tubules with sever interstitial nephritis with hemorrhage with detection bacterial colonies (Figure 7), in group 1.5% the lesions is less severity and the section appear normal except there was moderate interstitial nephritis (Figure 8).

Table 2: Biochemical characteristics of the G-ve and *S. aureus* isolates using VITEK® 2 Compact

Gram-negative isolates					*S. aureus* isolate		
Code	Reagent	*E.coli*	Result	Biochemical test	N.	Biochemical test	Result
9	BGAL	-	+	ADH1	8	ADH1	+
11	BNAG	-	-	BGAL	9	BGAL	-
17	BGUL	-	+	AGLU	11	AGLU	+
18	Dmal	+	+	PHOS	19	PHOS	+
23	Proa	-	-	PROA	23	PROA	-
33	SAC	-	-	Bgur	27	Bgur	-
36	CIT	+	+	ILTAK	39	ILTAK	+
43	NAGA	-	-	LAC	42	LAC	-
45	PHOS	-	+	Dmal	45	Dmal	+
48	LDC	+	+	NC6.5	50	NC6.5	+
57	BGUR	+	-	SAL	59	SAL	-
64	Ilata	-	+	Dtre	62	Dtre	+

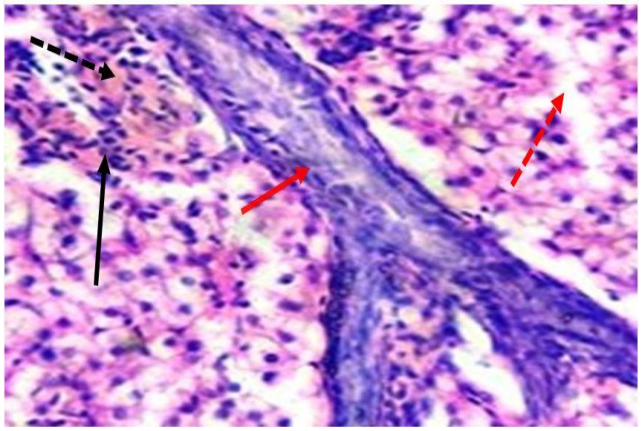

Figure 1: Microscopic examination of liver in infected fish *C. carpio* reveals fibrosis (red row), vacuolar degeneration (red dot row), infiltration of inflammatory cells (black dot row) with hemorrhage (black dot row), H&E 1*40.

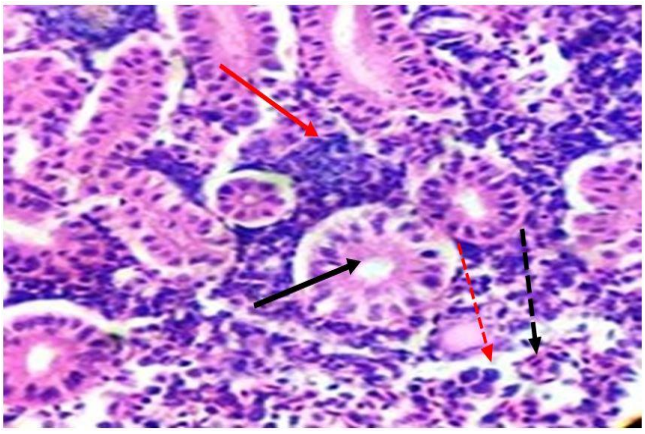

Figure 2: Microscopic examination of kidney in infected fish *C. carpio* reveals caogualative necrosis (black row), interstitial nephritis (red row) with hemorrhage (black dot row) with edema (red dot row), H&E 1.7*40.

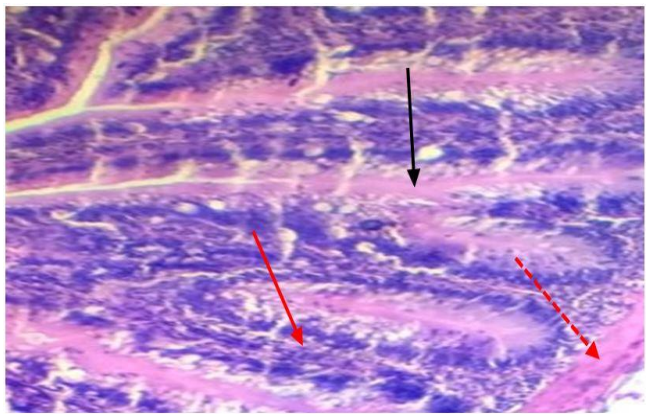

Figure 3: Microscopic examination of intestine in infected fish *C. carpio* reveals necrosis of muscular layer (red dot row), with adhesion of villi (red row) and loss the crypt with necrosis (black row) H&E 2.9*10.

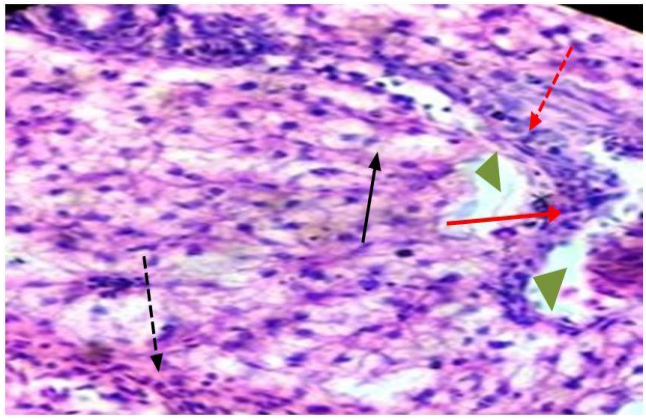

Figure 4: Microscopic examination of liver in infected fish *C. carpio* treated with 0.75% of *C. cassia* vacuolar degeneration (black row), infiltration of inflammatory cells (red row) with hemorrhage (black dot row) and fibrosis (red dot row) and edema (head row) H&E, 1* 10.

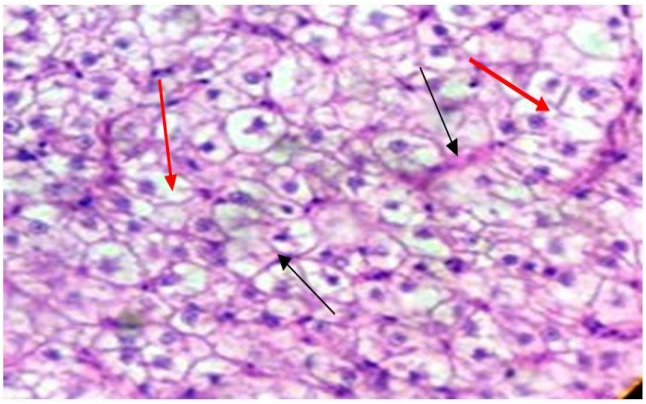

Figure 5: Microscopic examination of liver in infected fish *C. carpio* treated with 1% of *C. cassia* vacuolar degeneration (red row) and dilatation of sinusoid (black row) H&E, 1* 40.

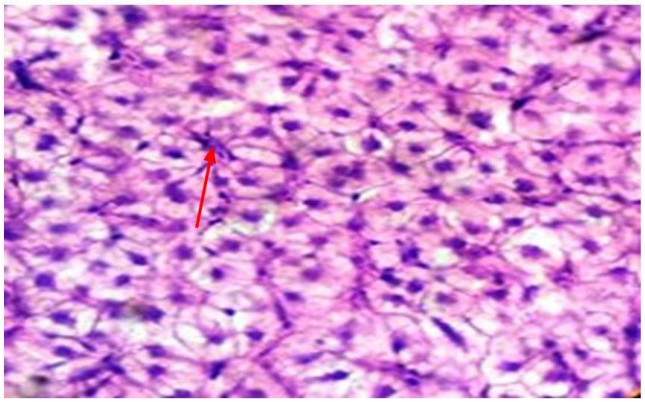

Figure 6: Microscopic examination of liver in infected fish *C. carpio* treated with 1.5% of *C. cassia* moderate infiltration of inflammatory cells (red row) H&E, 1* 40.

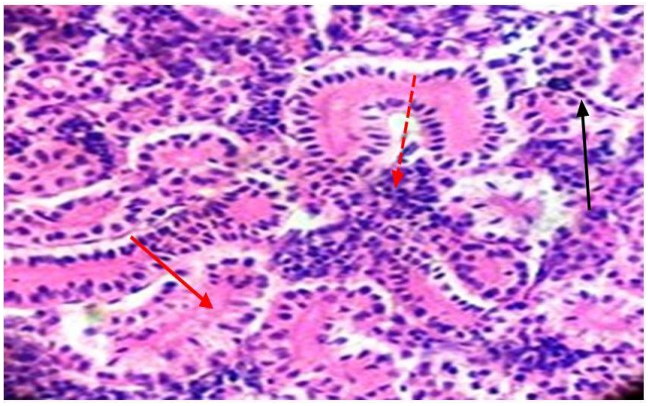

Figure 7: Microscopic examination of kidney in infected fish *C. carpio* treated with 1% of *C. cassia* coagulative necrosis in renal tubules (red row) sever interstitial nephritis with hemorrhage (red dot row), present colonies of bacteria (black row) H&E, 1* 40.

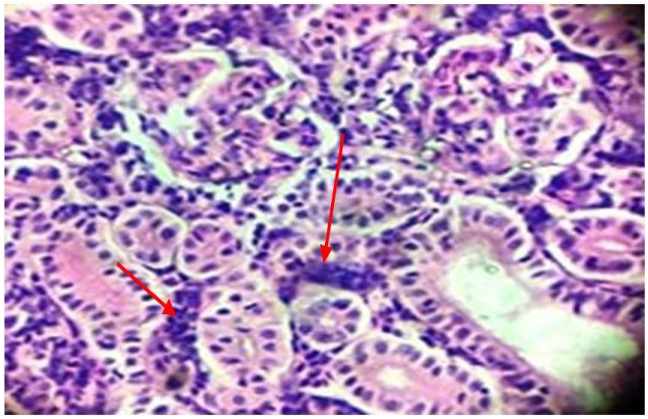

Figure 8: Microscopic examination of kidney in infected fish *C. carpio* treated with 1.5% of *C. cassia* exhibit moderate interstitial nephritis (red row) H&E, 1* 40.

There were no effects of *C. cassia* at 0.75% in repairing and healing the damage in illness fish intestine the microscopic examination reveals sever enteritis, hyperplasia of epithelial cells, adhesion of villi and loss crypt with necrosis at the apex of villi (Figure 9).

Lesions were less severity in group treated with 1% of *C. cassia* there was only moderate enteritis and less adhesion between villi with vacuolar degeneration in lamina properia (Figure 10).

more common effect of *C. cassia* at group treated with 1.5% the lesions less in severity the microscopic examination exhibit normal structure except there was slight infiltration of inflammatory cells with vacuolar degeneration see (Figure 11).

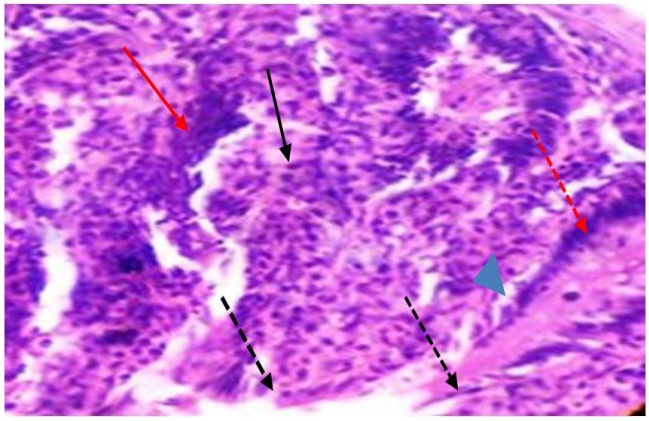

Figure 9: Microscopic examination of intestine in infected fish *C. carpio* treated with 0.75% of *C. cassia* exhibit sever infiltration of inflammatory cells (red row), hyperplasia of epithelial cells (red dot row), adhesion of villi (black row) and loss crypt (head row) with necrosis at the apex of villi (black dot row) H&E, 1* 10.

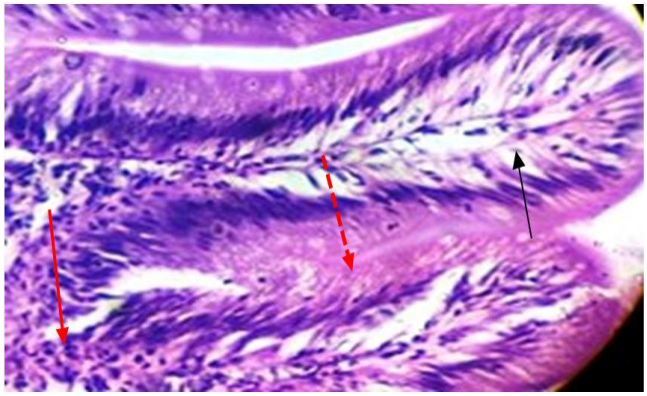

Figure 10: Microscopic examination of intestine in infected fish *C. carpio* treated with 1% of *C. cassia* moderate infiltration of inflammatory cells (red row) and less adhesion between villi (red dot row) with vacuolar degeneration in lamina properia (Black row) H&E, 1* 40.

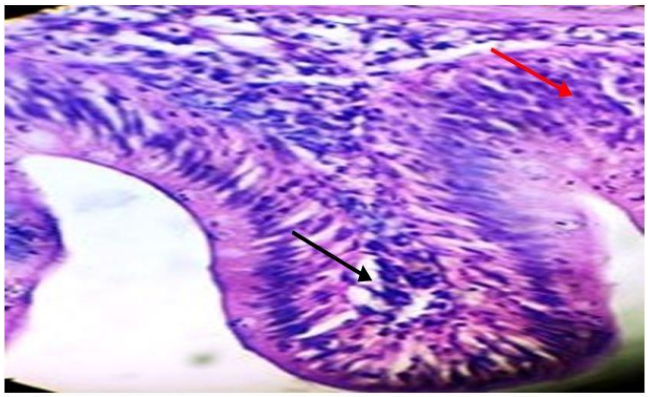

Figure 11: Microscopic examination of intestine in infected fish *C. carpio* treated with 1% of *C. cassia* slight infiltration of inflammatory cells (red row) with vacuolar degeneration (black row) H&E, 1* 40.

Discussion

Results in current study showed high percentage from both *S. aureus* and *E. coli*, followed *A. hydrophila*, and finally *K. pneumonia*, these results correspond to the results of zimbabwe study that shows that *E. coli* was the most isolate followed by *S. aureus* (23-25). Medicinal plants have properties as antiviral, antibacterial and anti-parasite (26), so they can be alternative the antibiotics to prevent infectious disease and also have been used for growth promoters and prevent stress, one of them was *C. cassia* which have properties of antioxidant and antibacterial (27,28). Histological changes consider as a good indicator for fish health status, so the result of present study shows that when illness fish treated with extracted rods *C. cassia* at 0.75, 1 and 1.5% for 8 weeks reveals the percentage 1.5% is more effective in repair and regenerative tissue damage in liver, kidney and intestine through it is important roles as antibacterial against *Staphylococcus* and *Aeromonas*, these result agreement with (29,30), also agreement with previous study of (31) who refer to it is effect of *C. cassia* as hepatoprotective and gastroprotective effects (15). *C. cassia stimulate* innate immunity as white blood cells which kill bacteria or by reactive oxygen and nitrogenous species which are toxic to bacteria (32,33), other immune defense mechanism is activation to lysosomal enzyme (34). *Cinnamomum spp*. is improve tissue is through it is activation to insulin-like growth factor (IGF-1) to increase metabolism and increase growth performance and enhance collagen and protein biosynthesis which increase body weight and maintain immune response and keep fish health (35).

Conclusion

More common effect of *C. cassia* at group treated with 1.5% the lesions more less in severity the microscopic examination exhibit normal structure except there was slight infiltration of inflammatory cells with vacuolar degeneration, so *C. cassia* have important role in regenerative tissue damage and keep fish in health status.

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Conflict of interest

No conflict of interest.

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تأثير إضافة القرفة على معالجة الإصابات المرضية لإصبعيات اسماك الكارب الشائع

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الخلاصة

هدفت هذه الدراسة إلى عزل وتوصيف المسببات المرضية التي أدت إلى نفوق إصبعيات اسماك الكارب في مفقس يانكي، وتأثير استخدام القرفة في علاج الإصابة. تم عزل نسبة عالية من المكورات العنقودية الذهبية والإيشريكيا القولونية والغازية القَوَوبة والكلِبسيلة الرئوية في كل من الكبد والكلى والأمعاء والتي أدت إلى حدوث تغييرات مرضية نسجية تمثلت بتليف الكبد وحدوث النخر التجلطي في الأنابيب الكلوية والتهاب معوي شديد. أضيفت القرفة إلى عليقة الأسماك المصابة وبالتراكيز ٠,٧٥ و١و ١,٥% ولمدة ثمانية أسابيع. اظهر الفحص النسجي أن التركيز ١,٥% كان أفضل تراكيز الإضافة العلفية للقرفة لإعادة ترميم وإصلاح الأنسجة المتضررة في الكبد والكلية والأمعاء. استنتج من هذه الدراسة انه بالإمكان استخدام القرفة كإضافات علفية وبتركيز ١,٥% لدورها في تحسين وترميم الأنسجة المتضررة والحفاظ على صحة الأسماك.