STUDY OF GROSS AND HISTOPATHOLOGICAL CHANGES IN *CYPRINUS CARPIO* FISH INFECTED WITH NON-HEMOLYTIC *STREPTOCOCCUS* PART 2: HISTOPATHOLOGY

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ABSTRACT

Sixty fishes of common carp (*Cyprinus carpio*) were obtained from the farm of Marine Science Center, University of Basrah. These fishes were divided into six groups (10 fishes/20 liters aquaria). One of these groups was injected intraperitoneally (I.P.) with an isolate of non-hemolytic *Streptococcus* at a concentration of 1 X 10^4 CFU/ ml. The other two groups were inoculated with 2.5 X 10^7 CFU/ ml of the same isolate via water pollution, one with and the other without skin abrasion. The other three groups were left as control and the experimental period was 35 days. The mortality rate was 100% in the I.P. inoculated group during the first week of the experiment, while nil in the other groups. The most important clinical signs of I.P. inoculated fishes were corneal opacity, erratic swimming, hemorrhage on the body surface especially in the base of fins. The other groups did not show any signs. Postmortem examination revealed general pathological changes of septicemia. Hemorrhage in the peritoneum, liver, kidney, gills, intestine, heart and brain of affected fishes. The kidneys were moderately swollen, enlarged spleen, pale liver as well as inflammation was noticed around kidney, heart and eye. Hyperplasia and necrosis of gills have been seen. Ascetic was also seen, while internal examination of other groups which were inoculated by aquarium with and without skin abrasion as well as their control groups did not reveal any obvious postmortem changes during the experiment. Microscopic examination revealed edema and inflammation of brain, eye exhibited hemorrhage and inflammatory cells infiltration, thickening of epithelial tissue of gills followed by necrosis and sloughing of these tissues. Degeneration and inflammation of myocytes and cardiocytes. Hydropic and fatty degeneration, congestion, hemorrhage and necrosis of hepatic cells. Glomerulonephritis and. depletion of hemopoitic tissue in kidney

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Apart of thesis for third researcher

In conclusion, *C. carpio* was experimentally infected with non- hemolytic *Streptococcus* via I.P. injection but it was not affected through contamination of water with or without skin abrasion

INTRODUCTION

Streptococcal infections of fishes became a major problem worldwide with the intensification of aquaculture (1,2,3). These diseases may be either chronic or acute. Acute outbreaks often occur during the warmer months of the year or when fishes are subjected to increasing stress. After an acute outbreak, a low-level chronic mortality can carry on for weeks or months with a small number of fishes dying every day (4).

Streptococcosis is transmitted via aquatic environment, that is, in the sediment and in the vicinity of fish's net pens (5). The bacteria released from dead and dying fishes being considered as the most important source of infection. Wild fishes may also carry the disease asymptomatically (6).

The quantity of bacteria required for the establishment of experimental infection (with CNS involvement) is considerably diminished if bacteria are loaded within macrophages substantiates the idea that *Streptococcus* can enter the CNS compartment in association with migrating monocytes, and it highlights the importance of macrophages as "Trojan horses" (7). Streptococcosis can be complicated by opportunistic motile Aeromonads and *Edwardsella tarda*, and the presence of parasites *Trichodina* and *Gyrodactylus* on the skin and gills (8).

Depending upon the species, affected fishes exhibit one or more of clinical signs (9). The pathology seen in the wild fish is more sever than that in the cultured fish. In the former, pathology was expressed as systemic disease with diffuse visceral hemorrhage versus, in the later, exudative meningitis accompanied by panophthalmitis. Sudden death of wild fish in the proximity of a farm could be used as an alert signal of streptococcosis (6). On histopathological study of infected culture tilapia, the bacterial dissemination was systemic. Abscess and granulomas were found to develop in the infected tissues as well as infiltration of bacterialaden macrophages (10).

The objective of the present study is to demonstrate the clinical sings, pathological and histopathological changes in common carp (*C. carpio*) experimentally with non-hemolytic *Streptococcus*.

MATERIALS AND METHODS

A total f sixty fishes of common carp (C. *carpio*) at average weight of 20-25 gm. had been obtained from the fish's farm of Marine Science Center. They were transported alive to the laboratory of Department of pathology, College of Veterinary Medicine, University of Basrah, by 4 Liters plastic jar. Non- hemolytic *Streptococcus* was isolated from the water of the same farm. The sixty fishes were divided into six groups at a density of 10 fishes/ 20 Liters aquaria, they were reared for about 35 days. The fishes were acclimated to the experimental conditions for 7 days from their arriving to the laboratory till starting with the treatment. Each aquarium was continuously aerated by air pump and maintained at 37 C°. These groups were treated as following:

The first group was intraperitoneally (I.P.) injected with non-hemolytic *Streptococcus* at a concentration of 1 X 10^4 CFU/ ml.

The second group was I.P. injected with normal saline and served as control for the first group.

A 2.5 X 10^7 CFU/ml of the same isolate was added to the aquarium of the third group.

The fourth group was used as control for the third group and was left without treatment.

The fifth group was subjected for abrasion through the removal of scales at both sides of fish body by a sterile scalpel. They were exposure to water contaminated by 2.5 X 10^7 CFU/ml of non-hemolytic *Streptococcus*.

The fishes of the last group were also subjected for abrasions and left in the aquarium without any other treatment and acted as control for the fifth group. (11).

Daily, one-third of the water was changed, dead fishes were removed and feces were siphoned off, and fish behavior was observed. Mortalities were recorded daily over 4 weeks period of the study (12).

Clinical signs, gross lesions and histopathological examination were performed. The histopathological sections were prepared from brain, eye, gills, skeletal muscles, heart, liver, kidney and intestine of dead and moribund fishes and examined by light microscope (13,14).

RESULTS AND DISCUSSION

On the first day of injection there were no obvious clinical signs and the fishes appeared with normal behaviors. On the second day, one of the I.P. injected fishes exhibited a unilateral cloudy eye (Figure 1). In spite of eye opacity food intake was not to be affected. On the third day post injection the fishes were noticed to swim near the border of the aquarium with erratic motion and swollen abdomen before death. Other fishes had decreased appetite and those with eye lesions became progressively blind and the skin color appeared darkened, hemorrhages were noticed within and around the eye as well as perforation of the cornea was

also observed. Anorexia, increasing numbers of blind fishes, these were seen with lethargic swimming near the sides of aquarium. Mortalities were started to increase at the third day post inoculation as shown in Table (1). All dead and moribund fishes were showed hemorrhages in the skin and head especially in the margin of mandibule and at the base of fins. The scales were weakened and easy to be removed. The total mortality of I.P. inoculated fish were 100% during seven days post injection. The second group was not exhibit any obvious signs. Fishes of other groups were subjected to the test bacteria in the water either with or without skin abrasion and their control groups did not reveal any clear clinical signs during the experimental period. The results of clinical signs in this study were in disagreement with that of (*10,15,16*) who stated that the most consistent clinical signs of infection with *Streptococcus* spp in trout is the development of exophthalmus and other serious eye lesions. Similar eye lesions were described in outbreaks of streptococcal infection in cultured turbot and in tilapia and channel catfish (12). On the other hand the result of this study was in agreement with that of (17) who mentioned that experimental infection of hybrid stripped bass and tilapias with *Strep. iniae*, did not reveal exophthalmia. (1) found that exophthalmia was a common feature of the disease in stripped bass, but the fish was failed to show this feature, while corneal opacity was exhibited in stripped bass, the blue fish, and sea trout. The degree of changes depending on the duration and severity of infection.

Both (3,18) suggested that the eye of many species of fish with *Streptococcus* spp. was affected the differences in eye lesions due to different species of streptococci, different species of fishes and experimental conditions.

In the present study *C. carpio* showed erratic motion, this result was in disagreement with that of (19) who did not record abnormal motion by per oral inoculation through food. While, this result was in agreement with that of (8) who observed erratic, raising and falling, and head-up swimming. Erratic motion is due to nervous system infection because non-hemolytic *Streptococcus* passes the brain-blood barrier and enters CNS (20).

All dead and moribund fishes showed external hemorrhages in the skin and head and at the base of fins in this study. The results were in agreement with that of (18) who mentioned that hemorrhage of skin were recorded in all cases of streptococcosis.

Body curvature was not seen in this study; therefore it was in disagreement with study (21) who recorded a body curvature in most of dead channel catfish with streptococcosis. Evans *et* (17) was reported that the body curvature in hybrid stripped bass and tilapia by nars inoculation. These deviations may be related to the rate of injection and the possibility of CNS involvement.

The macroscopic changes of present study were blood-tinged fluid in the body cavity, enlarged redden spleen, pale river and inflammatory process around kidney, heart and eye. These findings were in agreement with that of (10) who found that the internal lesions of streptococcosis were peritonitis, pale colored liver and epicarditis. (16,17) reported the same changes. Petechiae and ecchymoses in gills and other organs in this study were in agreement with that of (18) who reported these changes in *Streptococcus faecalis* infection in flounder fish.

In the present study histopathological examination of different organs of first grpoup characterized mainly by acute septicemic inflammation. Therefore these changes included congestion, hemorrhages and inflammatory cell infiltrations in different organs Fig. (2, 3, 4, 5). Necrosis and sloughing of epithelial cells of gills were also noticed in addition to degenerations such as hydropic degeneration of hepatic cells Fig. (6, 7, 8, 9, 10, 11). These results, in some way or another, were closely related to those findings of many authors indicated below.

(12) exposed three types of fishes to three different isolates of *Streptococcus* spp. Two of these isolates were induced microscopical meningitis, polyserositis of heart, liver, kidney as well as myocarditis. The third isolate was induced only mild granuloma in kidney of tilapia. The histological lesions caused by all three isolates in channel catfish consisted of meningioencephalitis, mild myocarditis but the lesions were not so sever. This indicates that *Streptococcus* lesions differ according to fish species. So *C. carpio* of present study had different changes compared with that of the above mentioned authors.

A histopathological study was made on an infection of *Streptococcus* in cultured tilapia, abscess and granuloma were found to develop in the infected orbital adipose tissue of the exophthalmic eyes, infiltration of bacteria-laden macrophage and granuloma formation were observed in epicardium, capsules of liver, peritoneum, stomach, intestine and brain (10).

The results of our study were in disagreement with of those histopathological changes. On the other hand, our results were in agreement with that of (21) who found that the histopathological findings of *Strep. iniae* infection in red drum produced a disease of systemic involvement characterized by multiple necrotic foci, and also with that of (22) who reported severe hyperplasia of gill tissues.

The histological changes observed in the fish suffering from *Strep.* D infection was typical of severe bacterial septicemia (23). The present study was exhibited closely related similarity with those findings.

It could be concluded that *Cyprinus carpio* intraperitoneally infected with non-hemolytic *Streptococcus* spp. Exhibited signs, gross lesions and histopathological changes of septicemic infection.

Table 1: Mortality of I.P. inoculated fishes during experimental period.

Days	No. of dead fish/day	**%**	Accumulative No. of dead fishes	**%**
1	0	0	0	0
2	0	0	0	0
3	1	10	1	10
4	1	10	2	20
5	2	20	4	40
6	3	30	7	70
7	3	30	10	100
14	-	-	-	-

Figure 1: Cloudy eye of *Cyprinus carpio* died 3rd day after I.P. injection.

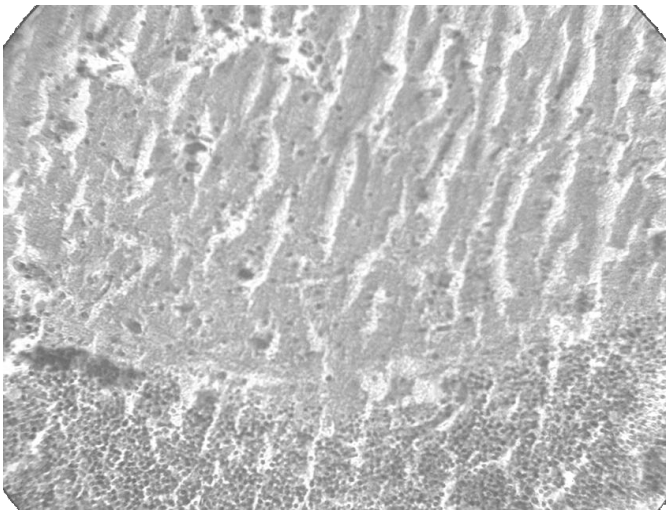

Figure 2. Brain: Edema(⟹), hydropic degeneration(⟷) &congestion(⟶). (H&E) x 10

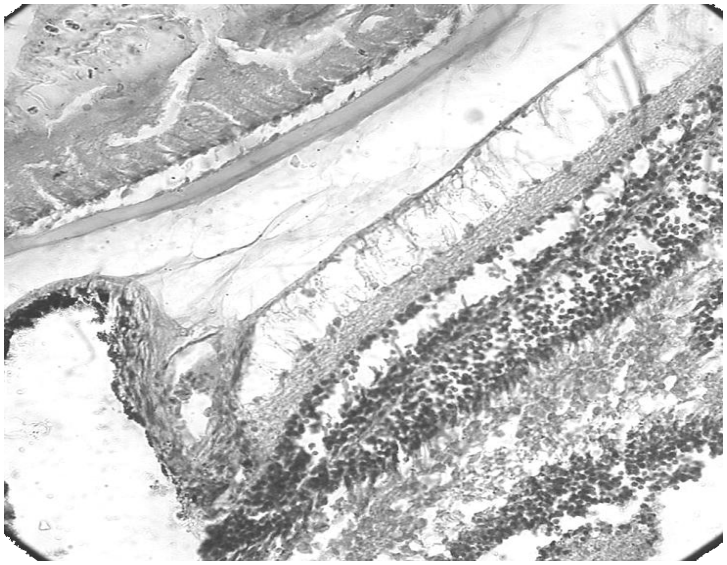

Figure 3. Eye: Hemorrhage(➡) and hydropic degeneration(➞) (H&E) x10

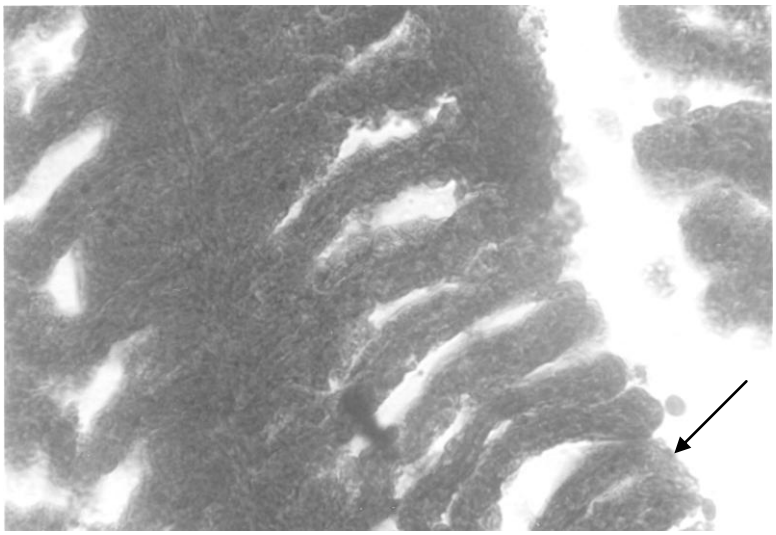

Figure 4. Gill : Thickening of the epithelial tissue of lamellae giving the appearance of a club (distal hyperplasia)(⟶) (H&E) x40

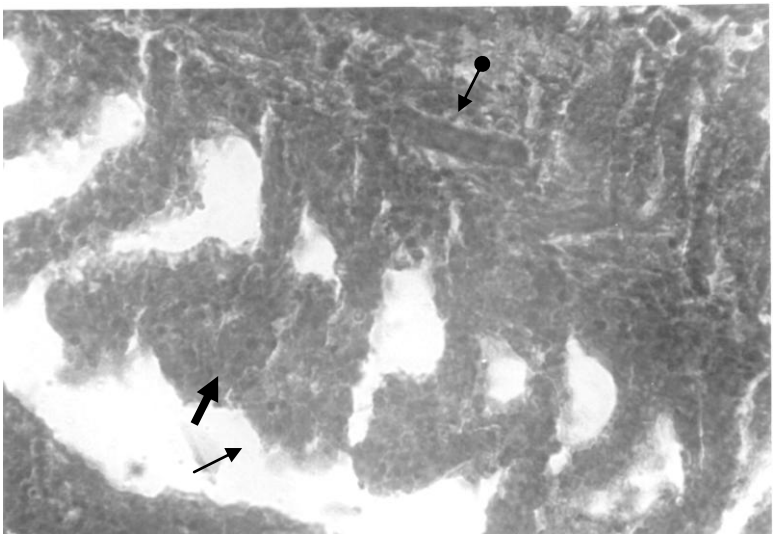

Figure 5. Gill :Inflammatory cell (⟶) ,hemorrhage in the primary lamella (●⟶) sloughing of necrotic tissue and hyperplasia(➡) . (H&E) x 40

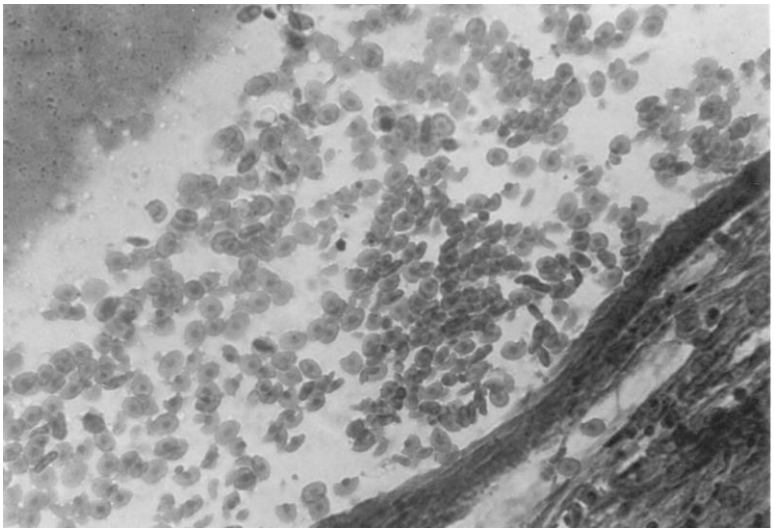

Figure 6. Gill : Sever hemorrhage in gill tissues (H&E) x 40 .

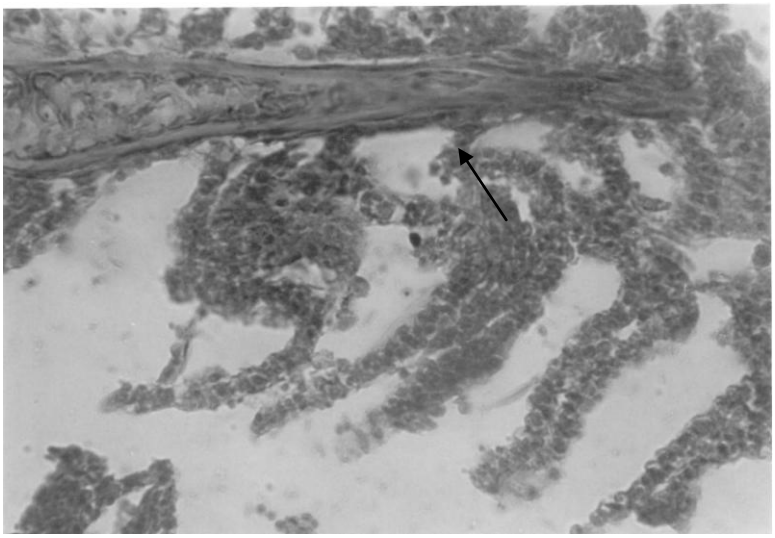

Figure 7. Gill .Sloughing of necrotic gill tissues(⟶) (H&E)x 40

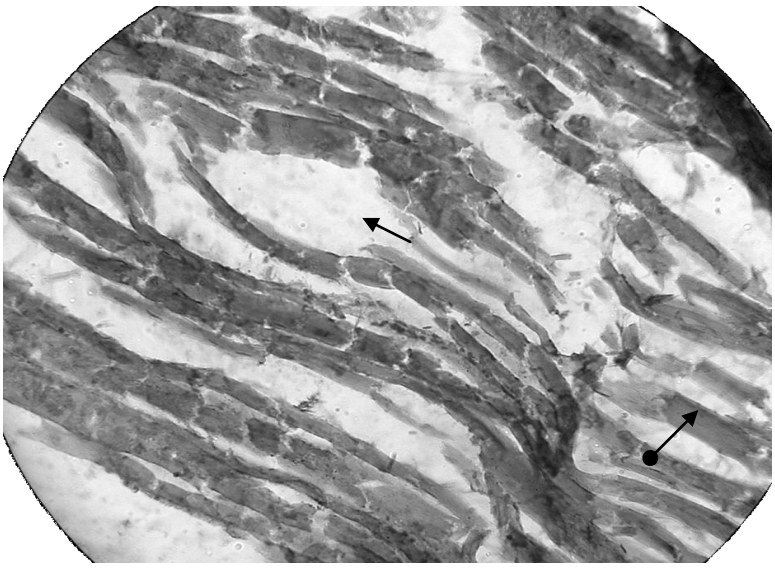

Figure 8;.Skeletal muscle: Edema(⟶), hydropic Degeneration(⟶). (H&E) x10

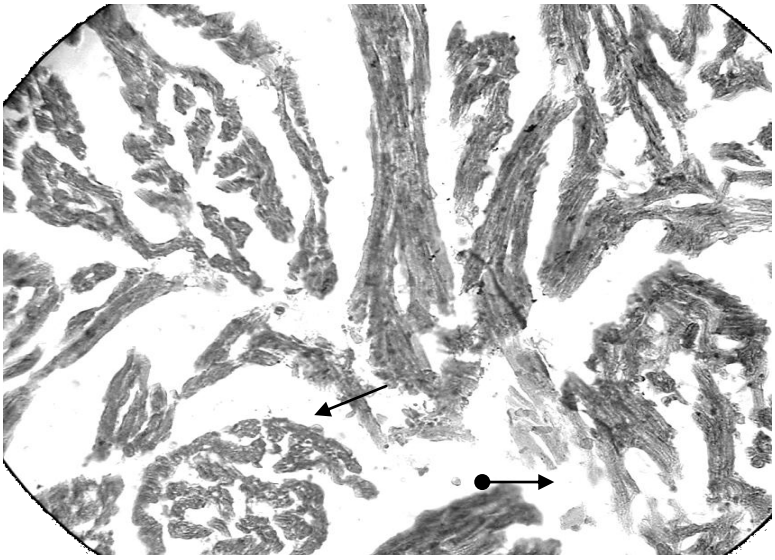

Figure 10;.Heart: Edema(→), degeneration of myocardial muscle(⟷) . (H&E) x10

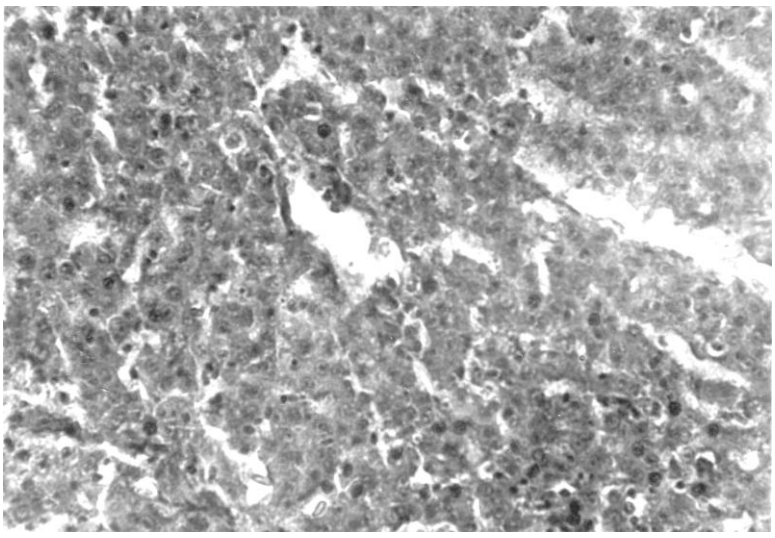

Figure 11;. Liver : Hydropic degeneration(→).Congestion and hemorrhage(●→) (H&E) x40

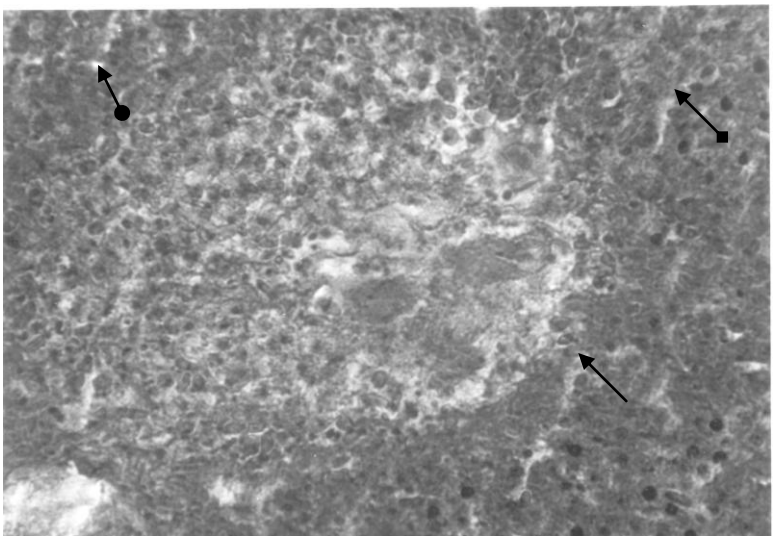

Figure 12; . Liver :Large irregular area of coagulative necrosis(⟶), vacuolization(⟶) & fatty degeneration(⟶).(H&E) x40

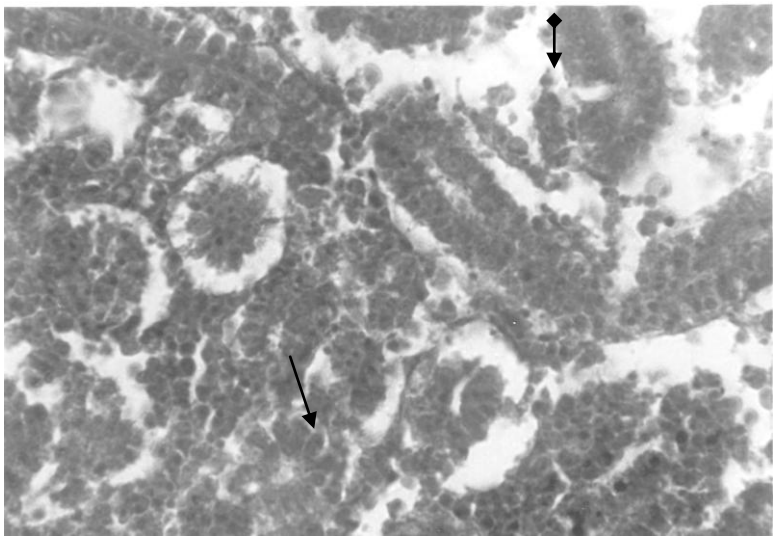

Figure13;. Kidney : Depletation of hemopoitic tissue( ) .Acute necrotizing glomerulonephritis and enlargement of glomeruli (⟶) .(H&E) x 40

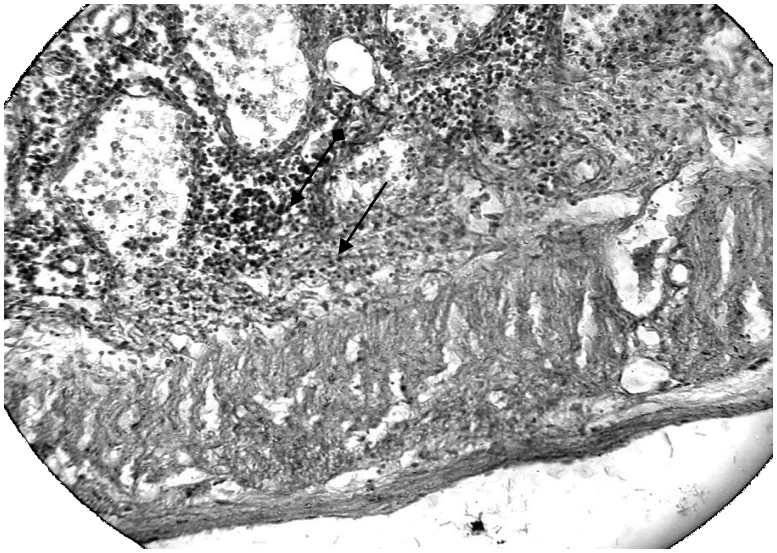

Figure 14;. Intestine : The proliferation of epithelial cells(⟷), hemorrhage &congestio

دراسة التغيرات العيانية والنسيجية في أسماك الكارب الاعتيادية المصابة تجريبيا" بجراثيم المسبحيات غير الحالة للدم
2- التغيرات النسيجية

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الخلاصة

جمعت 60 عينة من اسماك الكارب الاعتيادي المأخوذة بشكل عشوائي من محطة استزراع الأسماك التابعة إلى مركز علوم البحار – جامعة البصرة وتم إجراء التجربة في مختبر الأبحاث / كلية الطب البيطري / جامعة البصرة. قسمت العينات إلى ستة مجاميع بمعدل عشر سمكات لكل مجموعة . حقنت المجموعة الأولى بجراثيم المسبحيات غير الحالة للدم عن طريق الخلب بتركيز 1×10^4 جرثومة/مل، وتم تعريض مجموعتين عن طريق تلويث مياه الحوض بالجراثيم بتركيز 2.5×10^7 جرثومة/مل مع إزالة اسماك احد الحوضين وترك الأخرى بدون إزالة. استخدمت الأحواض الثلاثة المتبقية كمجاميع سيطرة. أظهرت العلامات السريرية على الأسماك المحقونة عن طريق الخلب عتمة شديدة في القرنية، قلة الشهية مع نزف في قاعدة الزعانف بالإضافة إلى الحركة العشوائية والبطنية التي ظهرت على الأسماك المصابة أثناء وجودها في الحوض. بعد إجراء الصفة التشريحية للأسماك الهالكة لوحظ وجود نزف في الخلب، الغلاصم، العين، القلب، الكبد، الكلية، الأمعاء، والطحال مع تضخم وتموت في أنسجة الغلاصم، تضخم الكلية والطحال والكبد وشحوب الكبد مع استسقاء في البطن. أما التغيرات النسيجية فأظهرت وجود فرط تنسج الغلاصم واندماج نهايات الخيوط الغلصمية، حدوث

التهاب لأنسجة الدماغ والعين والكلية، تنكس والتهاب عضلة القلب والعضلات الهيكلية إضافة إلى استنفاذ أنسجة الكلية، التنكس ألاستسقائي والدهني مع تنخر خلايا الكبد. كانت نسبة الهلاكات في اسماك الكارب المحقونة تجريبيا" بالخلب 100% في اليوم السابع من الحقن في حين لم تظهر أي هلاكات في بقية المجاميع طيلة فترة التجربة والبالغة 35 يوما". من هذه التجربة يمكن أن نستنتج إن اسماك الكارب الاعتيادي يمكن إصابتها تجريبيا" بجراثيم المسبحيات غير الحالة للدم من خلال الخلب فقط ، أما طرق الإصابة الأخرى فلا تسبب المرض.

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