

## Study of Cytopathological effect of B-hemolysin of *Aeromonas hydrophila*

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### ABSTRACT

In this study, the local isolate of genus *Aeromonas hydrophila* which isolated from local fresh water was used,. This bacterium was investigated to produce B-hemolysin, the haemolytic activity of hemolysin against different RBCs sources shown human RBC s was optimum for hemolysis with value 65 unit, while sheep and rabbit RBCs hemolysis were 55, 52 unit respectively. The Histopathological effects for hemolysin and bacterial suspension were study, it was found that the hemolysin more active on kidney tissue of injected mice and caused many changes comparatively with bacterial suspension include atrophy of renal glomerulus, degeneration of nucleus , vaculation of cytoplasm , PMNs filtration and few hemorrhage in kidney tissue.

### INTRODUCTION

*Aeromonas hydrophila* is gram- negative bacterium with straight rod - shaped, non- spore forming, oxidase- positive, glucose- fermenting, facultative anaerobic, and has optimal growth at 28°C but can also grow at the limits from 4°C to 37°C, (1) it belong to Vibrionaceae family (2); this bacterium can be isolated from different sources of foods, drinking water, and aquatic environments (3). It has different virulence factors assist in pathogenicity include haemolysin, aerolysin, cytotoxin, enterotoxin, cytotoxic enterotoxin, endotoxin lipopolysaccharide, outer membrane proteins, proteases, lipases, DNases, elastase , gelatinase and adhesins (4,5) .The main routes of exposure in humans are ingestion of contaminated foods and drinking water, or direct contact with recreational waters. However ; It caused many disease in humans include lesions ranging from gastroenteritis to septicaemia (6) gastroenteritis, peritonitis, cholangitis, soft tissue infections, septic arthritis, osteomyelitis, ocular

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infections, myositis, pneumonia, meningitis, hemolytic uremic syndrome , wound infections and urinary tract infection (7) *A. hydrophila* is also associated with cellulitis, an infection that causes inflammation in the skin tissue; it also causes diseases such as myonecrosis and eczema in people with compromised immune systems (8). Because the haemolysin consider important virulence factor for this bacterium for that, this study aimed to extraction ,partial purification and study their effect in animal tissues comparatively with bacterial suspension of *A. hydrophila* .

## MATERIALS AND METHODS

### Bacterial isolate

The *A. hydrophila* isolate was isolated from local water of Tiger River in Baghdad -AL-Jadria region among many bacterial genus, this bacterium was diagnosed by morphological characters and biochemical testes, and reconfirmation by API 20 E system. Bacterial strain was maintained on nutrient agar slant for 5 weeks and in L.B broth with 20% glycerol at -20°C for long time.

### Haemolytic activity

The haemolytic activity of the *A. hydrophila* isolate was determined by grown on blood agar plate assay; the pattern of haemolysis around the colonies on blood agar plates containing 5% (v/v) human blood were recorded after 24 hr incubation at 37°C (9).

### Extraction of Hemolysin

*A. hydrophila* strain was cultured in 100 mL of brain heart infusion broth supplemented with 0.3% of yeast extract (v/v) and incubated at 37°C for 48 hr in shaker incubator at 100 rpm ; the culture were harvested by centrifugation at 4000 rpm for 30 min at 4°C , the supernatant were immediately filter sterilized using 0.45 mm pore size filter , series of duplicate dilutions were prepared from bacterial supernatant by phosphate buffer saline (pH 7.4); the hemolytic activity was determined by added 1 ml of 1% RBC suspension (for human, sheep and rabbit) on each dilution in Microtiter plate and incubated for two periods 1 hr at 37°C and for other 1 hr at 4°C. the hemolytic activity was assayed by chosen the highest dilution that shown lyses completely for RBCs (10).

### Histological effect of bacterial suspension and B-hemolysin on Mice.

Three group of three male white mice (6 weeks age with 20-25g) were injected intraperitoneally, 1st group was injected with 1ml of bacterial suspension of *A. hydrophila*, 2nd group was injected with 1ml of partial

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purified enzyme, while 3rd group was injected with 1 ml of normal saline as control; all injected mice were left for 72hr, then killed and taking (kidney) among dissected organs and kept in 10% of formalin solution (10 ml formalin +90 ml of 0.9 % NaCl ) to preparation until histological sections.

### Preparation of histological sections

The histological sections were prepared according to Humson method (11), the organs were fixed by 10% formalin for 8 hrs, washed by tap water for several minutes and passed through a serial concentration of alcohol (50, 70, 80, 90 and 100%) for 2 hrs in each concentration, and then cleared by xylol for 1 hr and saturated with paraffin at 60°C for 3 hrs; the blocks were cut into several sections with 5-6 nm in thickness by using microtome; These sections were held on glass slides using Myer's albumin and left at 37°C to drying; Haematoxylin stain was used for 15 minutes ; washed

by tap water and acidic alcohol then washed by tap water again, after that Eosin stain was used for 15 second and then washed by D.W ; in order to hydration serial concentration of alcohol were used (70, 90 and 100%) for 2 minutes in each concentration ; Cleared by xylol again for 10 minutes then Canada balsam was used and covered by cover slide to examined under light microscope.

### RESULTS AND DISCUSSION

The bacterium of *A. hydrophila* that isolated from river's water in this study was able to haemolysis RBCs on blood agar plate , and shown ( $\beta$ -haemolysis pattern) completely clear zone surrounding their colonies with 4.8 mm in diameter that indicating extracellular haemolysin production, the human , sheep and rabbit erythrocytes were shown susceptible to the lytic action by *A. hydrophila* hemolysin with heamolitic activity value about 65 , 55 and 52 unite respectively (table1).

The haemolysis pattern of this bacterium in this study was agreement with other local report that shown  $\beta$ -haemolysis pattern different *A. hydrophila* isolates when grown on blood agar plate (12) and this results were compatible with Monfort and Balex observation they were note the human and sheep RBCs were lyses completely by hemolysin of different *Aeromonas spp* comparatively with other RBCs sources (10).

Table 1: Hemolytic activity of *A. hydrophila* B-hemolysin on different RBCs sources

Haemolytic activity unite	RBCs sources
65	Human RBCs
55	Sheep RBCs
52	Rabitt RBCs

The hemolyses of RBCs may attribute to believed that the hemolysin enzyme using calcium ions and then forming transmembrane pores in the lipid bilayers of erythrocytes (13). injection hemolysin of *A. hyrophila* in intraperitoneal mice were shown many histopathological changes in kidney includes Atrophy in glomerulus , vaculation of cytoplasm, degeneration of nucleus, dilation of tubules, inflammatory cells filtration (PMNs) in interstitial tissue and few hemorrhagic (fig 1-B); while the changes that occurred by bacterial suspension effect in kidney were less than hemolysin effect include PMNs filtration and vaculation in cytoplasm (fig1-C); comparative with control (fig 1-A). There are several studies were report about ability the B- hemolysin that produced by *A. hyrophila* to affect on different animal cells; Al-Vandi and his co-workers were observed many changes include Necrosis,

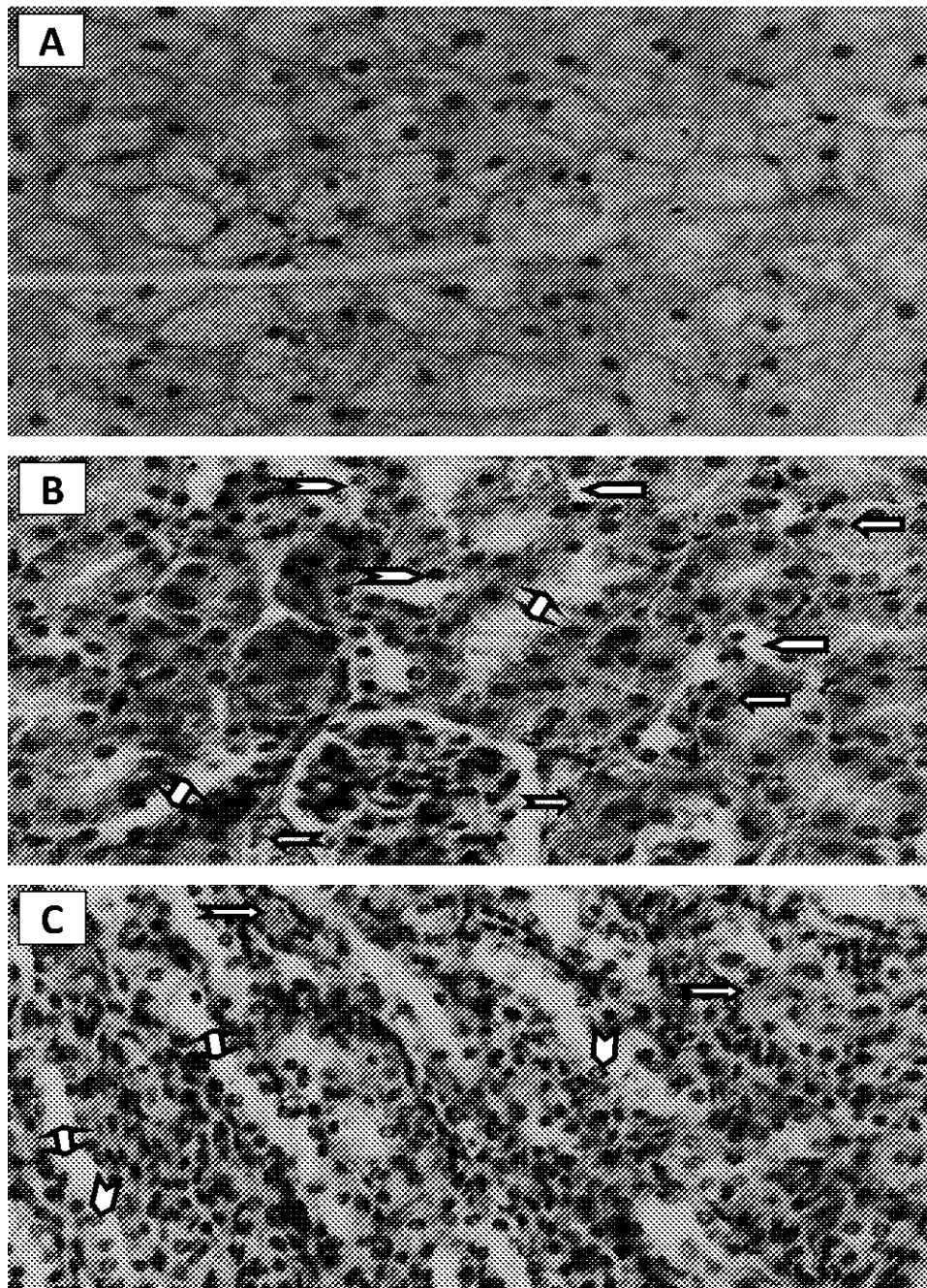


Fig 1: Cross section of mice kidney shows:.

**A:** Untreated kidney      **B:** Kidney treated with B-hemolysin of *A. hydrophila* shows Atrophy of glomerulus (◻), vacuolation of cytoplasm (◄►), degeneration of nucleus (⇒), filtration of PMNS (◁) and hemorrhage (⇨).

**C:** Kidney treated with bacterial suspension of *A. hydrophila* shows filtration of PMNS (◁), hemorrhage (⇨) and vacuolation of cytoplasm (◄►) X400 H&E.

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vaculation in cytoplasm and destruction of cellular membranes were occurred in different infected cells by *A. hydrophila* hemolysin were isolated from cases of diarrhea and domestic water samples (14) ; While Al-Rubai and his colleagues were document cytotoxic effect on RD and L20B cells was caused by hemolysin of clinical isolates *A. hydrophila* isolated from patients of diarrheic stool samples (12). Also, many of the histopathological changes were observed such as Necrosis, cytoplasmic vaculation , filtration fluids and then cellular death were documented in different cells such as liver, spleen and stomach tissues that infected by hemolysins that produced from many bacterial genus that close related with *Aeromonas* genus such as *Plasiomonas* (15, 16). From all above results , we conclude that *A. hydrophila* has ability to causes many histopathological changes in the kidney tissue of injected mice with hemolysin or bacterial suspension that may refer to caused Urinary tract infection in addition to diarrhea and intestinal infection ,also consider the B-hemolysin important virulence factor that has role in pathogenicity of this bacterium.

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## دراسة التأثيرات المرضية النسيجية للهيمولايسين بيتا المنتج من *Aeromonas hydrophila*

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### الخلاصة:

استخدمت في هذه الدراسة عزلة محلية من جنس *Aeromonas hydrophila* عزلت من المياه العذبة المحلية ، اختبرت قابلية هذه البكتريا لإنتاج الهيمولايسين بيتا ، إذ أظهرت فعالية التحلل للهيمولايسين بيتا ضد مصادر مختلفة من كريات الدم الحمر إن دم الإنسان هو الأمثل للتحلل بفعالية بلغت 65 وحدة ، بينما التحلل لدم الخراف والأرانب كانت مقدارها 52،55 وحدة على التوالي. كما درست التأثيرات النسيجية للهيمولايسين وعالق البكتريا، ووجد أن للهيمولايسين تأثيرا أكبر في نسيج كلية الفار المحقون وإحداث تغيرات عدة مقارنة بعالق البكتريا والتي تضمنت ضمورا في الكبيبات الكلوية وتنكس في انوية وتجوفا في السايوتوبلازم فضلا عن ارتشاح الخلايا الالتهابية مع نزف قليل في بعض مناطق النسيج.