Research Article

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Immune Response and Histopathological Changes of *Serratia rubidaea* in Mice

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Abstract

Serratia rubidaea is a zoonotic opportunistic pathogen that causes respiratory tract infection, septicemia, and encephalitis in humans and animals. The current study aimed to estimate the immunological response (IgG, IL-6by ELISA test and Delayed Type Hypersensitivity (DTH)-Skin Test and histopathological lesions (intestine, liver, kidney, and spleen) by using twenty-four Swiss mice divided into three equal groups (8/each).The first group(G1) as control negative given PBS (pH 7.2) 0.1ml S/C, the second group(G2) immunized by killed whole cell antigen *Serratia rubidaea* (KWCA SR) (1.2 $\times 10^9$CFU ml) S/C and the third group(G3) immunized by sonicated whole cell antigen *Serratia rubidaea* (SWCASR) (1000 µg/ml) S/C. A booster dose was given after 14 days of immunization to the G2 and G3 the same as the immunizing dose. At 21 and 28 days of immunization, IgG and IL-6 concentrations were increased significantly ($P \leq 0.05$) in the groups G2 and G3 compared to the negative control group. DTH-Skin Tests of G2 and G3 groups showed increased induration diameter after 24 and 48 hours. and a decrease at 72 hours. Histopathological lesions showed mild to moderate changes in immunized groups compared with the positive control group in all organs. In conclusion, it was found that KWCASR and SWCASR induce humoral (IgG) and cellular immune responses(IL-6,DTH), which reduce the pathological lesions in the immunized mice.

Keywards: *Serratia rubidaea*, ELISA, IgG, IL-6, DTH, sonicated antigen.

الاستجابة المناعية والتغيرات النسجية المرضية لجرثومة *Serratia Rubidaea* في الفئران

الخلاصة:

تعد جرثومة *Serratia Rubidaea* من الامراض الانتقالية و الانتهازية حيث تسبب عدوى الجهاز التنفسي وتسمم الدم والتهاب الدماغ في كل من الإنسان والحيوان. هدفت الدراسة الحالية إلى تقييم الاستجابة المناعية (IgG,IL-6) وفرط الحساسية من النوع المتأخر والتغييرات النسجية المرضية وذالك باستعمال أربعة وعشرين فأراً سويسرياً مقسمة إلى ثلاث مجاميع متساوية (8/ لكل مجموعة).المجموعة الأولى (G1) هي مجموعة السيطرة السلبية بحقنها المحلول الملحي الفسلجي المتعادل ذو الاس الهيدروجيني 7.2، المجموعة الثانية (G2) تم تمنيعها بواسطة مستضد الخلية الكاملة المقتولة(KWCASR) Serratia Rubidaea بجرعة × 1.2) 10^9CFU ml)والمجموعة الثالثة (G3) تم تمنيعها بواسطة مستضد الخلية الكاملة المكسرة بجرعة Srratia Rubidaea (SWCASR) 1000 µg/ml بعد ذالك أعطيت جرعة معززة في يوم14 من التمنيع للمجموعتين G2 و G3بنفس جرعة التمنيع. أظهرت النتائج في يوم 21 و 28 من التمنيع، زادت تراكيز IgG و IL-6 بشكل ملحوظ ($P < 0.05$) في المجموعتين G2 و G3 مقارنة بمجموعة السيطرة السلبية. أظهر اختبار DTH للمجموعتين G2 و G3زيادة في قطر التصلد بعد 24 و48 ساعة. وتنخفض عند 72 ساعة. أظهرت التغييرات النسجية تغيرات خفيفة إلى متوسطة في المجاميع الممنعة مقارنة بالمجموعة السيطرة الإيجابية. في الختام، وجد أن KWCASR و SWCASRيحفزان الاستجابات المناعية الخلطية (IgG) والخلوية(DTH) ، مع تقليل التغييرات النسجية المرضية في الفئران الممنعة.

Introduction

Serratia species is a Gram-negative rod-shaped facultative anaerobic bacteria belonging to the family Enterobacteriaceae (1). There are ten species of *Serratia* recorded: *S. marcescens, S. Liquefaciens, S. Proteomaculans, S. Grimesii, S. Plymuthica, S. Rubidaea, S. Odorifera, S. Ficaria, S. Fonticola,* and *S. Entomophila* (2,3). It is often poorly understood as zoonotic opportunistic bacteria in both veterinary and human medicine. In humans, bacteria can be isolated from blood, wounds, bile, feces, and respiratory tracts (4,5,6). Additionally, there is also a report about the presence of this bacterium in a wound infection after a horse bite and intestinal content of carp. (7,8). *Serratia* is an extracellular bacterial that is shielded from infection by opsonizing antibodies and frequently resolves after a significant portion of the bacterium has been phagocytized by mononuclear and polymorphonuclear leucocytes(9).

Cytokines and chemokines, through their regulation of host inflammatory responses, play a crucial role in dictating the course of infection (10), and interleukin IL-6 is involved in controlling the multiple host responses such as inflammation, acute phase responses, hematopoiesis, and B-cell development into the plasma cells which produce antibodies (11), and IL-6 is also previously known to protect against infectious diseases (12).

Trypsin and some other trypsin-like serine proteases activate PAR-2, which in turn modulates immune and inflammatory responses; Serralysin, a protease derived from *Serratia* is thought to be involved in the pathogenesis of infection; The three processes are comprise the cellular immune response phagocytosis, autophagy, and apoptosis and facilitate by circulating hemocytes (13). In vertebrate animals use phagocytosis to destroy small foreign organisms, while in invertebrate animals use autophagy to clear their bodies from pathogens (14).

There is much lack information about the immunogenicity of *S. rubidaea* antigens, so this study conducted to assess the immune responses of killed whole cells *S. rubidaea* antigen (KWCASR) and sonicated whole cells *S. rubidaea* antigen (SWCASR) against the *S. rubidaea* infection in mice.

Materials and Methods

Ethical Approval

Ethical approval of this study was granted according to the local committee of Animal Care College of Veterinary Medicine / University of Baghdad under the license number P-G/650 in 24/3/2024.

Isolation and identification of bacteria

Serratia rubidaea was isolated from cattle fecal samples at Baghdad city. The samples were inoculated onto multiple agar plates (Nutrient, MacConkey, and Chrome), and incubated at 37°C for forty-eight hrs. (15). The morphological, biochemical, Vitek 2 compact system and PCR methods were used to identify the isolated bacteria to the species level (16).

Antigens preparations

Serratia rubidaea killed whole-cell antigen (KWCASR) and sonicated whole-cell antigen (SWCASR) was created after harvesting the bacteria from nutrient agar. They were centrifuged for 20 min. at 3000 rpm, rinsed three times with PBS (pH7.2), and then formalin (0.5%) was added. They were incubated for two hours at 37 °C before leaving overnight at 4 °C. Following three cleanings with PBS (pH7.2), the bacterial suspension was centrifuged at 3000 rpm for 20 min. to get the KWCASR , resuspension and maintained in the

frozen state. Also, to obtain SWCASR, the suspension was subjected to one min. for sonication and one min. for rest, using an Ultrasonicate set to operate at a rate of 15 kHz/sec on ice. The result was finally filtered using a Millipore filter (0.45µm) after centrifuging the sonicated bacterial solution for 20 min. at 3000 rpm (17). The suspension was then kept in a frozen state at -20 °C. Bradford's method was employed to measure the protein concentration for the SWCASR (18), whereas the McFarland tube method was used to determine colony forming unit/ml for the KWCA of *S. rubidaea*.

Experimental study and sample collection:

Twenty-four healthy Swiss mice of both sexes aged between 6-7 weeks, weight between 15-20g were divided into three groups (each group 8 mice) as follows: The first group - G1(Control negative) given PBS (pH 7.2) 0.1 ml S/C, the second group- G2 immunized by of KWCASR 0.3ml (1.2×10^9CFU/ml) S/C and the third group- G3 immunized by SWCASR 0.3ml(1000 µg /ml) S/C. After 14th days of immunization booster dose was given to the second and third groups. Blood samples were collected from G1, G2, and G3 at 0, 21, and 28 days post-immunizations, and sera were adequate for estimating the IL-6 and IgG concentrations by ELISA kits (Elabscience, China) the Delayed Type Hypersensitivity(DTH)-Skin Test was done on day 18th of immunization. DTH-skin test involved taking 0.05 ml of the SWCASR that had been previously prepared and injected intradermal (ID) into the right hind footpad of mice, while the left hind footpad of all immunized groups received 0.05 ml of sterilized PBS (pH 7.2) and measurements of skin induration was performed after 24, 48, and 72 hours by using vernier caliper (19).

Infectious dose

The infectious dose was prepared according to the McFarland tubes, by using 3 mice from 3 groups above, who were infected by a virulence strain of *S. rubidaea* (3.0×10^8CFU / ml) 0.1 ml orally, after 7 days of given the infectious dose, three mice of each three groups were sacrificed to obtain the vital organs(liver, spleen, kidney, and intestine) to study the histopathological changes.

Histopathological examination

After 21 days of post-immunization of mice, all animals were infected with 3.0 $\times 10^8$CFU/ml orally. They were monitored every 6 to 8 hrs. for 7 days for clinical signs and mortality, after 7 days later (28 days of Immunization), experimental animals were sacrificed for histopathological lesions and vital internal organs (liver, kidney, spleen, and intestine), and these organs were sliced into pieces (1cm) and fixed in a 10% formalin solution, paraffin block (1cm) , ascending and descending ethyl alcohol, xylene and sectioning for slides preparation and histopathological examination (20).

Statistical analysis

The SAS (Statistical Analysis System) application was utilized to determine how the various factors affected the study parameters. To compare between means, the Analysis of Variation-ANOVA test was employed at least significant difference –LSD (21).

Results and discussion:

Colony morphology and bacterial identification:

Colonies of *S. rubidaea* have been a red color on nutrient agar, whereas they are dark pink to red on MacConkey and chrome agars (Figure, 1).

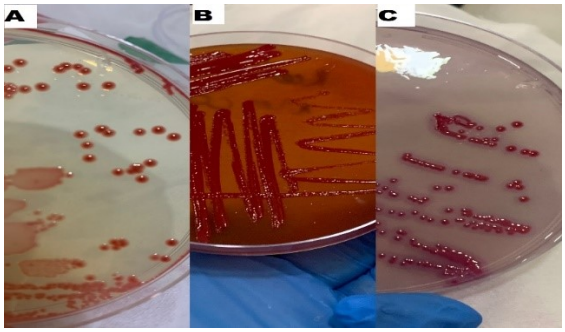


Figure (1): *Serratia rubidaea* on (A)Nutrient, (B) MacConkey and (C) Chrome agars.

By Vitek 2 compact system was given *S. rubidaea* 99% probability and the isolate was registered in Gen Bank under accession No. OR757107.1. The protein concentration of the sonicated antigen was 4 mg\dl, this investigation greater than the results of (22), who used the same method of sonication whole-killed *Serratia marcescens* and the WKS-SM protein concentration of 1 mg/ml.

IL-6 concentration:

The concentration of IL 6 in the immunized groups (KWCA and WCSA) was significantly (P≤ 0.05) increased at 21st and 28th days of immunization. The immunized group (KCSASR) had the highest concentration (1014.54 and 994.30 pg/ml) respectively, followed by the SWCA Group (997.28 and 957.53pg/ml) respectively, (Table, 1). These findings may be explained by the significance of the innate immune system in host defense against invasive microbial pathogens through particular recognition mechanisms; additionally, normal gastrointestinal flora maintains close and constant contact with immune cells, and the stimulation that results is necessary for immune system maturation (23). Additionally, the generation of inflammatory cytokines, which block neutrophils' and macrophages' ability to neutralize IL-6 in infected laboratory animals that die (24). Also, IL-6 plays a key role in the humoral immune response, promoting the production of antibodies by antibody-secreting cells (ASCs) and inducing B-cell differentiation into plasma cells (25). Our results were in agreement with (26) who observed that sonicated antigen (WCSA-S) of Serratia was able to promote stronger humoral and cellular immune responses against *S. marcescens* infection.

Table (1): IL-6 concentration in immunized mice by ELISA test.

Groups	0days	21days	28days
G1(C-) PBS	130.93±0.62 A a	132.39±1.16 A c	132.24±0.67 A c
G2(KCSA SR)	135.20±0.49 B a	997.28±2.05 A b	957.53±3.18 A b
G3(SWCASR)	134.64±1.06 C a	1014.54±1.34 A a	994.30±2.91 B a
LSD	12.38		

Means with a different small letter in the same column are significantly different (P≤ 0.05)

Means with a different capital letter in the same row are significantly different (P≤ 0.05)

IgG concentration :

A significant (P≤ 0.05) increase in IgG concentration of immunized groups (KWCASR and WCSASR) was demonstrated on days 21 and 28 after immunization compared to the control negative group and they had the highest antibody concentrations (85.14 and 93.78pg/mL) respectively on day 28. (Table, 2). Our findings indicated a significant (P≤0.05) increase in IgG concentration within days in all immunized groups, while (27) prepared antigens from sonicated *Salmonella typhimurium* (KWCSA-ST) and Lactobacillus acidophilus (KWCSA-LBA) to assess the synergistic effect on interleukins production (IL-2, IL-4) and immunoglobulin-G (IgG), which were evaluated using the ELISA, the

group immunized with KWCSA-ST 1000 µg/ml and KWCSA-LBAg 1000 µg/ml had significantly (P≤0.05) higher serum concentration of IgG at 35th day post-injection than 10, 21, and 49 days post-injection. Following this, there is a relationship between the creation of IL-6 and an increase in IgG concentration. Initially, IL-6 was identified as a cytokine that promotes the synthesis of antibodies in a B cell line (28).

Table (2): IgG concentration in the immunized mice by ELISA test.

Groups / Days	0	21	28
G1(C-) PBS	18.44±0.55 A a	18.46±0.65 A c	17.75±0.70 A c
G2(KWCASR)	17.12±0.74 C a	41.94±0.56 B b	85.14±0.44 A b
G3(SWCASR)	18.11±0.86 C a	47.40±0.58 B a	93.78±0.88 A a
LSD	1.98		

Means with a different small letter in the same column are significantly different (P<0.05)

Means with a different capital letter in the same row are significantly different (P<0.05)

Delayed type hypersensitivity- Skin Test:

The results of the DTH (Skin Test) showed high diameter measurement of induration in the immunized groups and the KWCA group showed an increase of induration in the diameter at 24 and 48 hrs., followed by a decrease at 72 hrs. (2.98mm, 3.94mm, and 3.02mm) respectively. While the results of SWCA were shown at 24 hrs. 3.50 mm, at 48 hrs. 4.10mm, and at 72 hrs. 3.82mm with a significant difference (P≤0.05). (Table, 3). This decrease was attributed to mononuclear infiltration into the lesion site within the 24–48-hour period. Memory T-cells have been demonstrated to be necessary for this reaction since the response is controlled by the CD4+ and CD8+ fraction. Th1 (T helper) cells are known to release TNF-β, IL-2, and IFN-γ. For cell-mediated inflammatory responses such as delayed hypersensitivity and the macrophage activation with Th1 cells mainly serve as helper cells (29).

Table (3): DTH-Skin Test induration diameters (mm) in immunized mice.

Groups / Hours	24	48	72
G1(C-) PBS	1.32±0.004 A c	1.32±0.006 A c	1.31±0.004 A c
G2(KWCA)	2.98±0.003 C b	3.94±0.003 A b	3.02±0.005 B b
G3(SWCA)	3.50±0.01 C a	4.10±0.004 A a	3.82±0.006 B a
LSD	0.017		

Means with a different small letter in the same column are significantly different (P<0.05)

Means with a different capital letter in the same row are significantly different (P<0.05)

Histopathological changes:

Histological changes shows different histopathological changes in the vital organs. In G1 (control-positive infected *S. rubidaea* 3.0 ×10^8CFU/ml /orally), the liver shows portal triad with marked perivascular cuffing of lymphocytes with marked vascular degeneration of hepatocytes (Figure, 2), kidney shows severe interstitial nephritis with interstitial infiltration of leukocytes and mild tubular necrosis with cast formation (Figure, 3). kidney with severe interstitial nephritis with interstitial infiltration of leukocytes (Figure, 4), spleen appears to deteriorate of splenic architecture with severe edema and proliferation of megakaryocytes (Figure, 5) and intestine shows marked thickening of villi and

marked hyperplasia of lining cells with severe infiltration of mononuclear leukocytes (Figure, 6). In G2 (KWCA): the liver showed disarrangement of hepatic cords with severe necrosis of hepatocytes and sinusoidal dilation with infiltration of leukocytes (Figure, 7). Glomerulonephritis revealed marked generalized vascular degeneration with necrosis of lining cells of renal tubules with focal necrosis with infiltration of leukocytes (Figure, 8). Deterioration of splenic lymphoid follicles with tissue depletion marked proliferation of megakaryocytes (Figure, 9). The intestine showed marked hyperplasia of lining cells of intestinal glands with little infiltration of mononuclear leukocytes with normal enterocytes (Figure, 10). In G3 (SWCA): the liver section revealed disarrangement of hepatic cords with severe sinusoidal dilation with infiltration of MNLs and necrosis of hepatocytes (Figure, 11). kidney showed tubular dilation and marked desquamation of lining cells of renal tubules mind interstitial edema with infiltration of leukocytes (Figure, 12). The spleen showed deterioration of splenic architecture with severe edema tissue depletion and proliferation of megakaryocytes (Figure, 13). The intestine showed necrosis of intestinal glands with marked infiltration of mononuclear leukocytes (Figure, 14). Furthermore, the histological lesions found correspond with the findings of studies conducted by (30) and (31) that investigate the histopathological changes that occur after being immunized with *Serratia marcescens* antigens. The white pulp in the kidney was depleted, whereas the liver showed congestion of dilated sinusoids and accumulation of mononuclear cells in the portal region surrounding blood vessels. Following direct proliferation in the lymphoid tissue attached to the mucosa, Serratia species proceed to the liver and spleen via the mesenteric lymph nodes. This triggers a wide-ranging immune response that results in the synthesis of humoral, cell-mediated, and secretory immunoglobulin A (IgA) antibodies (30,32).

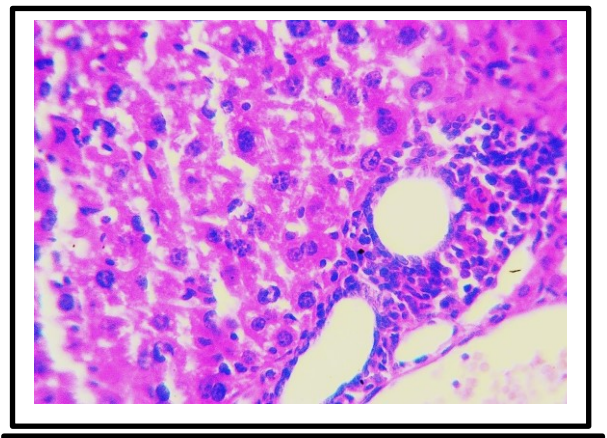


Figure (2) G1: Liver section show portal triad with marked perivascular cuffing of lymphocytes with mark vascular degeneration of hepatocytes .H&E.40x

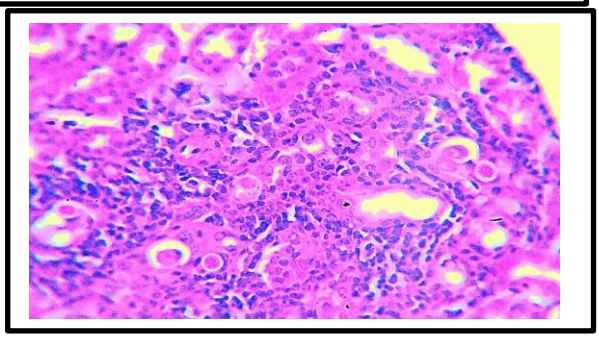
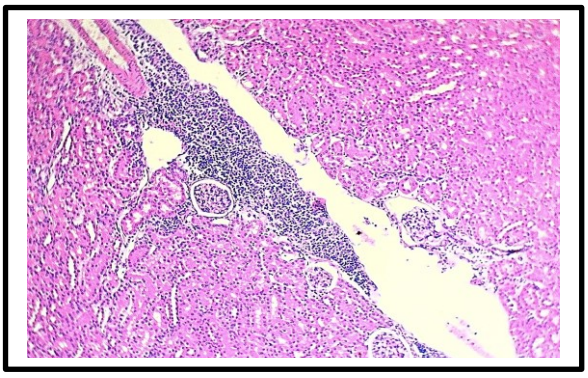


Figure (3) G1: Section of the kidney show severe interstitial nephritis with infiltration of leukocytes and mild tubular necrosis with tubular cast formation .H&E.40x.



Figure(4) G1:Kidney with severe interstitial nephritis.H&E.10x

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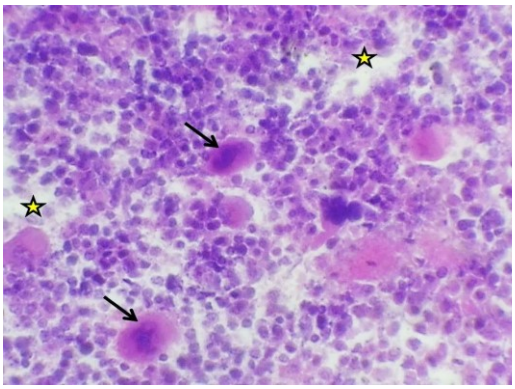


Figure (5) G1: Section of spleen deterioration of splenic architecture with severe edema (Asterisk) mark proliferation of megakaryocytes (arrows) .H&E.40x

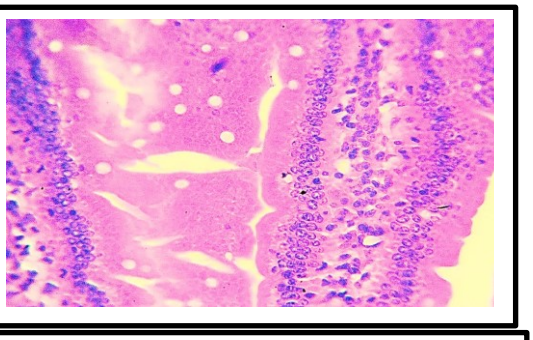


Figure (6) G1: Section of intestine mark thickening of villi with hyperplasia of lining cells.H&E.400x.

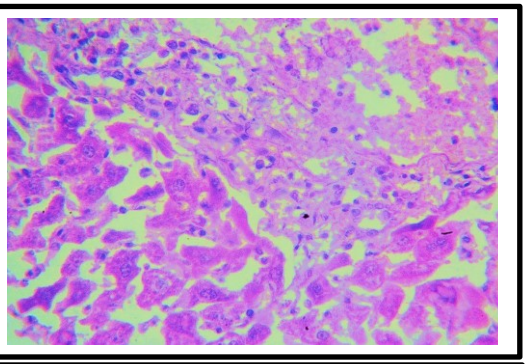
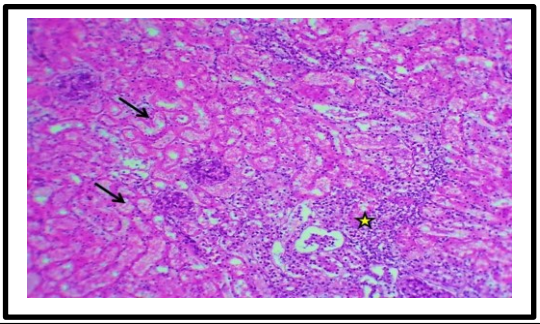


Figure (7)G2:Disarrangement of hepatic cords with severe necrosis of hepatocytes, sinusoidal dilation with infiltration of leukocytes .H&E.400x.



Figure(8)G2:Kidney show nephritis reveal mark generalize vascular degeneration with necrosis of lining cells of renal tubules (arrows) with focal necrosis with infiltration of leukocytes (asterisks) .H&E.10x.

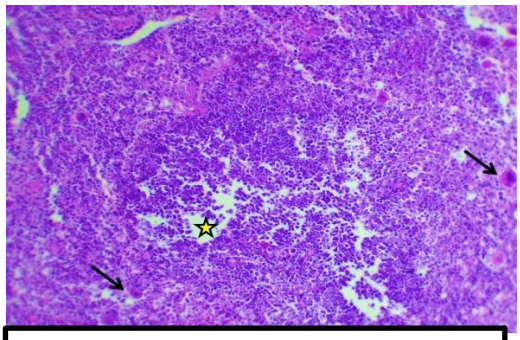


Figure (9)G2: Deterioration of splenic lymphoid follicles with tissue depletion (Asterisk) mark proliferation of megakaryocytes (arrows) .H&E.10x.

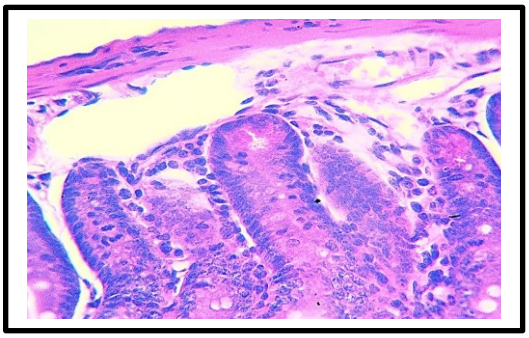


Figure (10)G2: Show mark hyperplasia of lining cells of intestinal glands with little infiltration of mononuclear leukocytes with normal enterocyte .H&E.40x

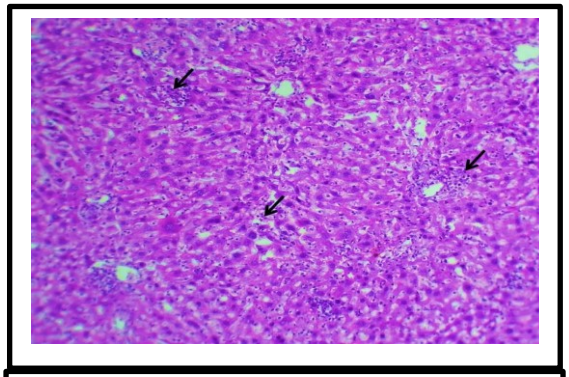


Figure (11)G3: liver section revealed disarrangement of hepatic cords with severe sinusoidal dilation with infiltration of MNLs (Arrows) and necrosis of hepatocytes .H&E.10x.

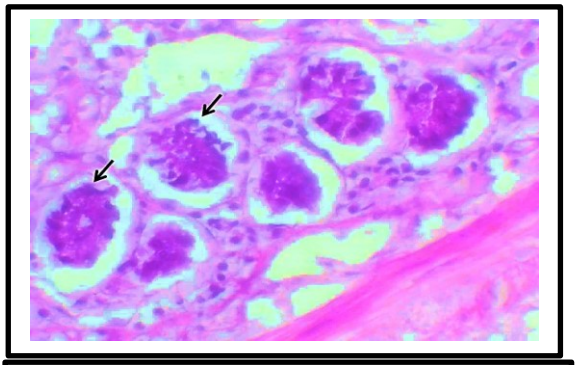


Figure (12)G3: Kidney section show tubular dilation and mark desquamation of lining cells of renal tubules (arrows) mind interstitial edema with infiltration of leukocytes. H&E stain.40x .

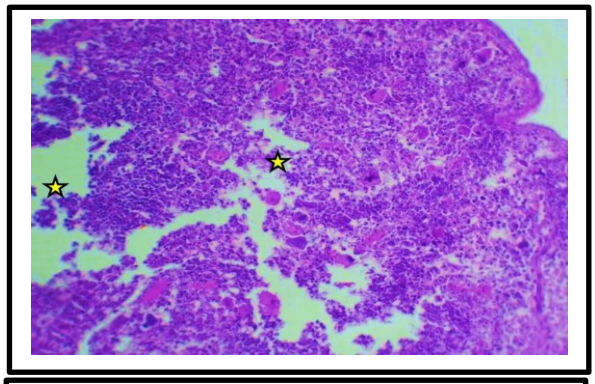


Figure (13)G3: Deterioration of splenic architecture with severe edema and tissue depletion (Asterisk) mark proliferation of megakaryocytes (arrows) .H&E.4x.

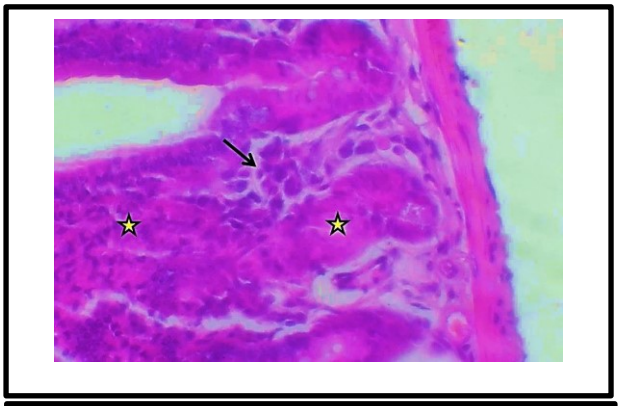


Figure (14)G3: Intestine show necrosis of intestinal glands (asterisk) with mark infiltration of mononuclear leukocytes (arrows) .H&E.40x

Conclusion:

In conclusion, KWCASR and SWCASR were observed to promote humoral (IgG) and cellular immune responses with a decrease in the histopathological effects of *Serratia rubidaea* in mice.

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Conflict of interest:

The authors declare no conflicts of interest regarding the publication and or funding of this manuscript.

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