Pathological study on mice intestine experimentally infected with Shiga toxin-producing *E. coli* O26

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Abstract

Mice infected with STEC O26 (2.5×10^8 C.F.U/ml) to study the pathological changes in intestine due to infection. The gross pathological changes in the small and large intestine were characterized by variable degrees of hyperemia with engorgement semisolid stool was present in the proximal half and fully formed feces in distal colon. The histopathological changes were characterized by, intestine with all stages of sloughed, eroded and complete necrosis of the villi also diffusion together, necrosis the epithelial cells lining the intestinal glands, diffuse inflammation, and crypt abscesses. Moreover, increase in the goblet cells as well as depletion at late stage. In conclusion, The experimental mice infection with STEC O26 induced a significant pathological changes in intestine.

Keywords: STEC, O26, *E.coli,* cytokines, mice, duodenum, colon, cecum, PAS, Alcian Blue.
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دراسة مرضية على امعاء الفئران المصابة تجريبيا بالاشيريشيا القولونية الفارزة لسموم (stx) العترة O26

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الخلاصة

لقد تم استخدام الفئران لاستحداث الاصابة بالبكتريا الفارزة للـ(stx) النمط العتري O26 وبالجرعة (2.5×10^8C.F.U/ml) وذلك لدراسة التغيرات المرضية العيانية والمجهرية للامعاء بسبب الاصابة. اظهرت نتائج الفحص المرضي العياني للامعاء الدقيقة (الاثني عشر) والغليضة (الاعور والقولون) بدرجات متفاوتة من التغيرات واشتملت احتقان دموي، وتكون البراز بشكل اقرب للسائل في النصف العلوي من القولون مع تكون كامل للبراز في الجزء الاخير من القولون. التغيرات المرضية النسجية تميزت الامعاء بانسلاخات وتنخرات في الطبقات الظهارية للامعاء، مع تنخر الزغابات المعوية واتحادها مع بعضها، وانتشار الخلايا الالتهابية في جميع طبقات النسجية للامعاء، مع خراجات متعددة في داخل الطبقة الظهارية، مع فرط في تنسج وافراز مخاط الخلايا الكأسية في المراحل الاولية للاصابة، ونضوبها في المراحل المتأخرة للاصابة. في الختام اظهرت الدراسة ان العترة O26 مسبب مرضي مهم لاصابة الاطفال والاصابة التجريبية لها في الفئران سببت تغيرات وافات مرضية مهمه في الامعاء.

Introduction

Shiga toxin-producing *E. coli* (STEC) infection have been described in a wide range of both domestic and wild animal species, but their natural pathogenic role has been demonstrated only in young calves, pigs and dogs. The cattle being recognized as the major reservoir for human infections (1, 2, 3). Human infection with STEC usually causes serious diseases (3, 4), such as bloody diarrhea, hemolytic uremic syndrome, hemorrhagic colitis,

thrombotic thrombocytopenic purpura, fever, vomiting, and possible death (5, 6, 7). Patients who develop fever usually also develop HUS at some stage of HC, several complications may arise from STEC range from inflammation of the gastrointestinal to gangrene with peritonitis and sepsis, to rectal prolapse, coma, hemiplegia, pancreatitis and seizures (8, 9). STEC serotypes O26 has prime importance in causing disease in humans (10, 11). Also it has been isolated from calves and lambs with diarrhea (1, 12, 13). This strain is a virulent to young calves (14). It has been linked to both human and animal illness for more than 25 years (15, 16). It was accounted for the largest proportion of infections among STEC (17, 18), and it was ranked among the top of STEC serogroups isolated from individuals with sporadic illness reported to the CDC between 1983 and 2002 (19). Interestingly, STEC O26 possesses many of the similar virulent factors as STEC O157, but it has possess additional ability to infect animals (20, 21, 22). O26 appears to survive in bovine intestinal tract, rather than that other food animals (11). Serotype O26 has been reported from healthy cattle and cattle, mainly calves, with diarrhea, also outbreak accured in Canada in cattle with HC (23, 24), and in Spain (25). Outbreaks associated with O26 in the U.S. have been linked to lake water (Minnesota, 2001), a daycare (Nevada, 2005 and Iowa, 2007), blueberries and strawberries (Massachusetts, 2006), raw milk (Washington, 2010), and ground beef (multistate outbreak, 2010) (26). In 1997, Japan (27). In 2000, an outbreak in Germany (28). In 2007, in Denmark (29). On 2016, the CDC announced two outbreak in multi-state of USA, the initial, larger outbreak was first detected in 11 state in October 2015 with the outbreak strain STEC O26 were reported from ill people were hospitalized, and in December, 2015, a second outbreak of strain of STEC O26 was identified in other three states (30). Shiga toxins are involved in diarrhea by killing absorptive villus tips on epithelial cells, resulting in an imbalance in intestinal absorption and secretion (8, 31). The bloody diarrhea is attributed to the role of stx on endothelial cells, thrombotic microangiopathy, and the lesions on small blood vessels in the gut (32).

Materials and Methods

- **Bacteria Preparation and Harvest:** STEC O26 which taken from child suffering from diarrhea, showed positive result in PCR analyses to the fallowing genes (*wzx*$_{O26}$, *stx1*, *stx2*, *eaeA* and *EHEC hlyA)* which has previously isolated in Ph.D. dissertation program. With a sterile wire-loop, some bacteria were streaked into a petri-dish containing MacConkey agar. A colony from harvested and cultured on an EMB agar to confirm that the colony was *Escherichia coli*. Tryptic soy broth (100 ml) to calculate bacteria according to (33).
- **Mice:** one hundred seventy mice ranged from 8 to 12 weeks old which obtained from the (Iraqi Center for Cancer and Medical Genetics Research), fed clean boiled water and laboratory chow ad libitum were used in this study. After 1 day of streptomycin treatment mice (6mg/ml) according to Krystle and Alison, (2011), mice was starved from 18 to 24 h from food then divided randomly into two groups: "infected group" (85 mice) which were inoculated orally with infectious dose of STEC O26 (2.5×10^8 C.F.U/ml) and "control group" (85 mice) which were inoculated orally with phosphate buffer saline. Blood samples were collected and sera were separated from the infected mice (25 mice from each group) at 3, 14 and 21 days after induced infection and kept at (-20 ˚C) for immunological tests. Skin test were done on (10) mice from each groups at 21 days post infection.

- **Histopathological examination:** Pathological effect in intestine at 3, 7, 14, 21, and 27 days. Specimens of intestine were taken from internal organs, the tissue were fixed in 10% buffer formaldehyde solution immediately after washing with normal slain. After 72 hrs of the fixation, the specimens were washed with tap water and then processing was routinely done with a set of upgrading alcoholic concentration from 70% to absolute 100% for 2 hrs in each concentration to remove water from the tissues, then clearance was done by xylol, then the specimens were infiltrated with liquid paraffin wax at 58-60°C on two stages, 2 hrs each; blocks of specimens were made with paraffin wax and refrigerated finally they were sectioned by rotary microtome at 4-6 μm. All tissues were stained with either one of the following stains: Mayer's Hematoxylin and Eosin routine stain for identification (general features) and Masson's trichrome, Congo red, Alcian blue (ph. 2.0) and PAS (periodic acid Schiff) according to (34). Then histopathological changes were observed under light microscope.

Result

- **Grossly:** The intestine of control mice showed proximal colon with fully formed pellets feces. In contrast, infected mice, showed semisolid stool which was present in the proximal part of colon, with pellets forming in in the distal part of colon only. And cecum was appeared to be significantly engorged in infected mice. The infected mice intestine at days 14 showed engorgement, enlarged, edematous colon. The small intestine was normal grossly. Fig. (1).
- **Microscopically**

1- Day 3: The duodenum of infected mice showed changes include truncated villi with fusion; proliferation with infiltration of inflammatory cells in submucosa edematous lamina propria. Fig. (2). The cecum from infected mice revealed infiltration of lamina propria with inflammatory cells infiltration. Fig. (3). Moreover, increase in goblet cells of the colon with inflammatory infiltrates; with bulging mucus droplets oriented towards the lumen and thickening of the submucosa. Fig. (4).

2- Day 7: Histopathological section in the intestine of infected mice at day 7 post infectious with STEC O26; duodenum revealed presence of truncated villi fused together with complete necrosis of their lining epithelial cells; the villi were short and the lamina propria was edematous with infiltration of inflammatory cell; Fig. (5). The cecum from infected mice did not show a cellular components at the lamina propria; Fig. (6). The colonic epithelial layer of infected mice were short, thin, and irregular, and Goblet cells at epithelial cell with inflammatory exudate of and polymorphonuclear leucocytes, RBCs, fibrin, and mucus erupting from a small ulcer in the colonic mucosa this picture is compatible with pseudomembranous enterocolitis. Fig. (7).

3- Days 14: At days 14 the major of intestinal section showed similar pathological changes as those seen in period (7 days); duodenum showed villus sloughing or ulceration, eroded with crypt with abscesses and sloughing of its epithelial cells. Fig. (8). The cecum showed erosive changes and depletion of goblet cells. Fig. (9). The colon showed erosive changes of epithelia were noticed with sever hemorrhage. Fig. (10).

4- Day 21 and 28: Cecum at day 21 showed epithelial layer erosive with regenerative epithelial. Fig. (11). Colon at same period of post infection showed regenerative process of epithelial layer with a mild inflammatory cells infiltration and submucosal thickening. Fig. (12).

Fig. (1) Macroscopic changes in the intestines of mice, at days 3, 7 and 14. (A)The intestine of control mice showed proximal colon with fully formed pellets feces In contrast, infected mice, showed semisolid stool which was present in the proximal part of colon, with pellets forming in in the distal part of colon only. And cecum was appeared to be significantly engorged in infected mice (B). The infected mice intestine at days 14 showed engorgement, enlarged, edematous colon(C).

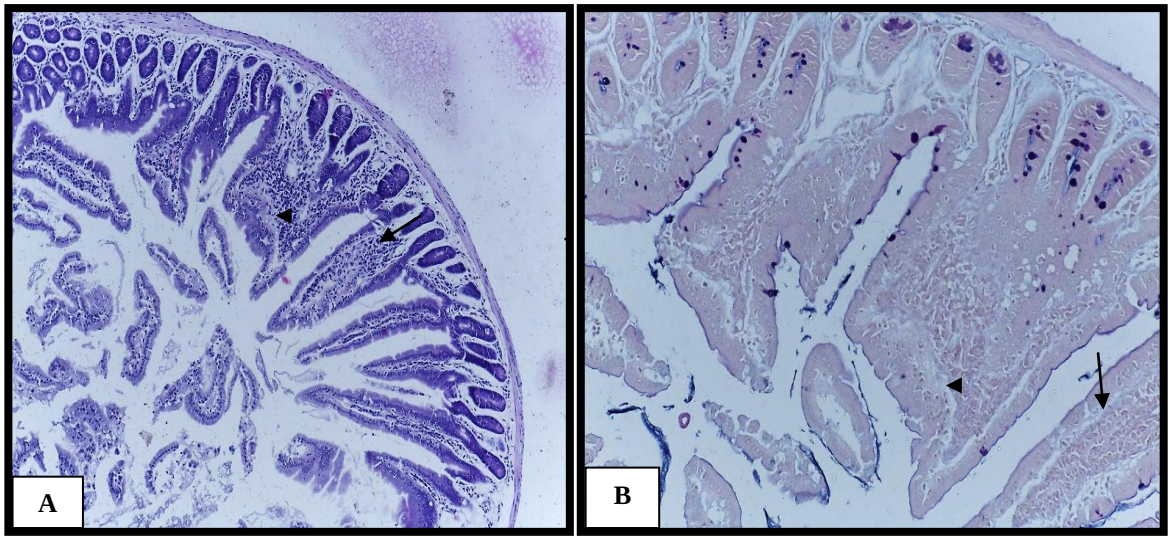

Fig. (2) Histopathological section in duodenum of infected mouse at day 3 showed inflammatory infiltration within villi (arrow) with fusion and edema in the lamina propria (arrowhead) (A) H and E stain 100X (B) Alcian blue stain 200x.

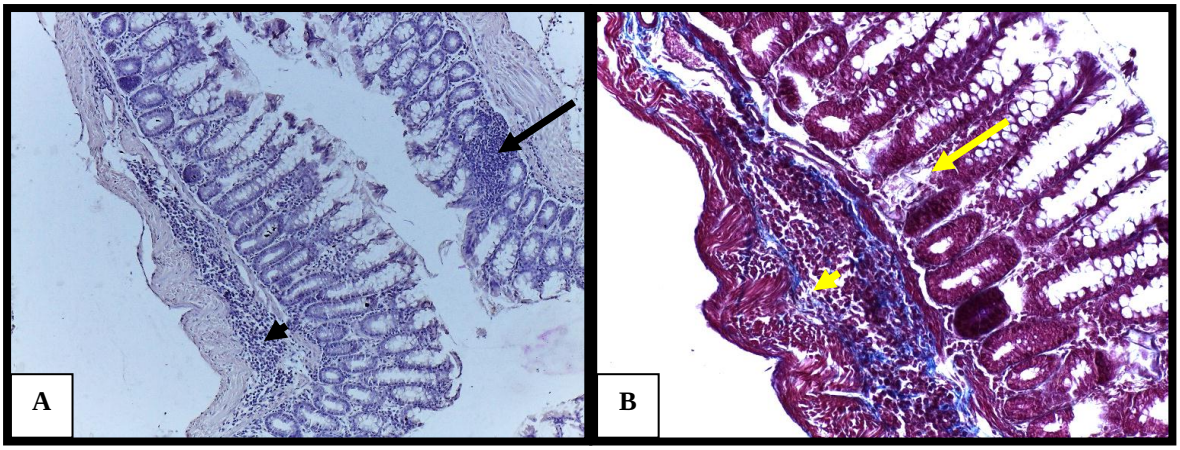

Fig. (3) Histopathological section in cecum of infected mouse at day 3 shows aggregation of inflammatory cells (lymphoid tissue hyperplasia) and edema in the lamina (arrow) propria and sub mucosa (arrowhead) (A) H and E stain 100X (B) Alcian blue stain 200x

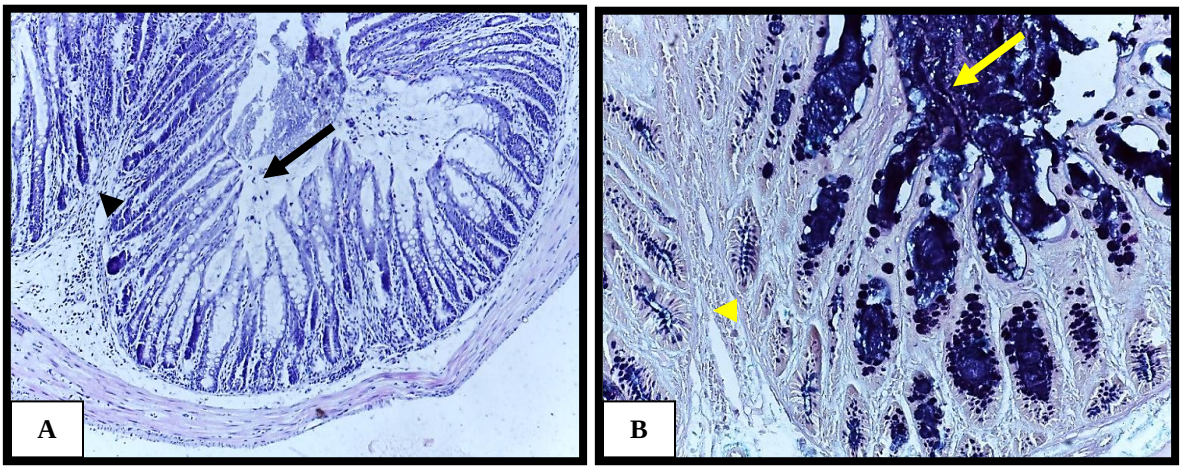

Fig. (4) Colon at day 3 with Alcian blue staining of goblet cell mucin revealed increased numbers of goblet cells and ballooning in infected mice colon with bulging mucus droplets oriented towards the lumen (arrow) edematous submucosa and infiltration of inflammatory cells (arrowhead) (A)H and E stain 100X(B)Alcian blue stain 200x.

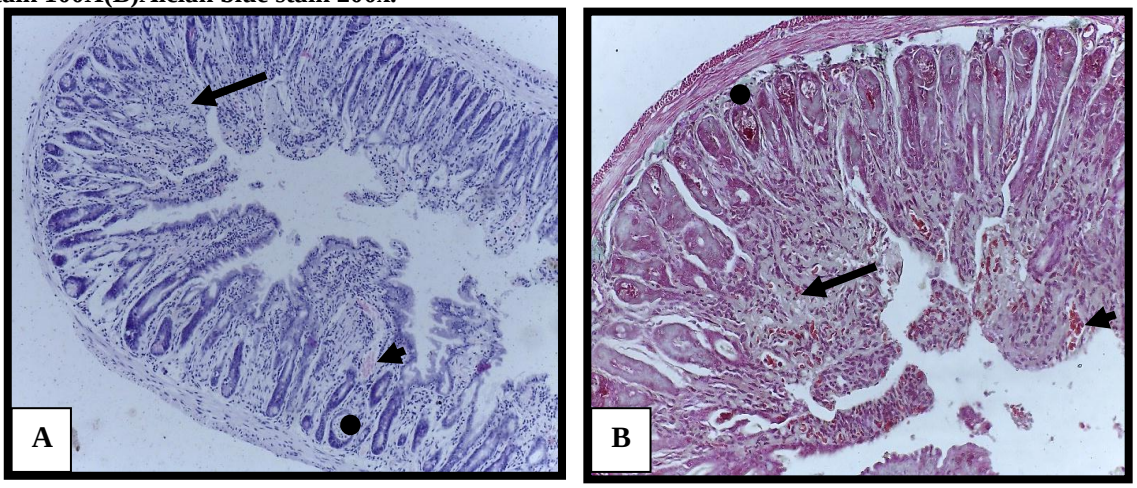

Fig. (5) Duodenum from infected mice at day 7 shows villi fused together (atrophy of epithelial cell) with complete necrosis of lining epithelial cells (arrow) and with increased in the numbers of intestinal inflame cells and congestion of blood vessel (arrowhead) within lamina propria with crypt abscesses (point). (A)H&E stain 100X (B) Masson's Trichrome stain 200X.

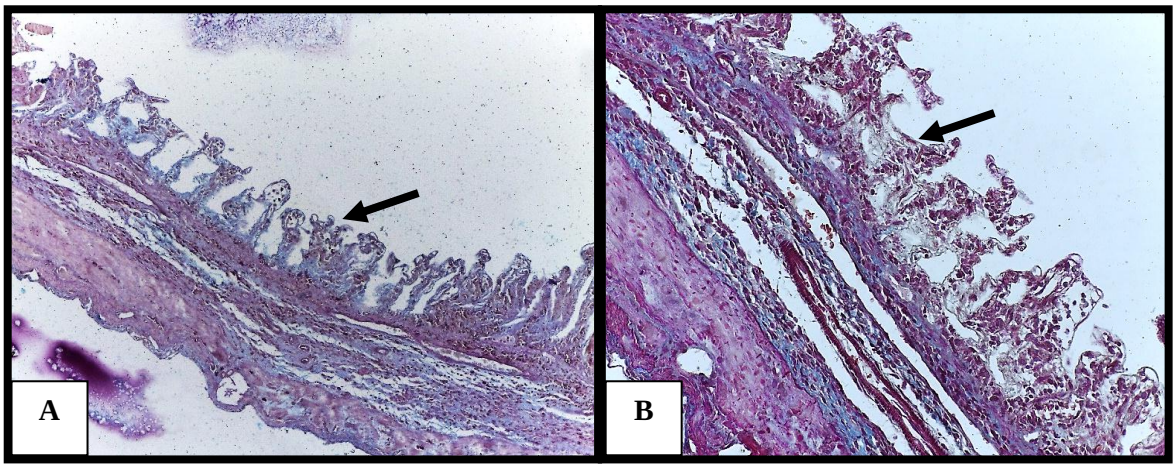

Fig. (6) The cecum from infected mice at day 7 showed absence of cellular components were at the lamina propria of cecal epithelia (arrow). (A) H and E stain 100X (B) Masson's Trichrome stain 200X.

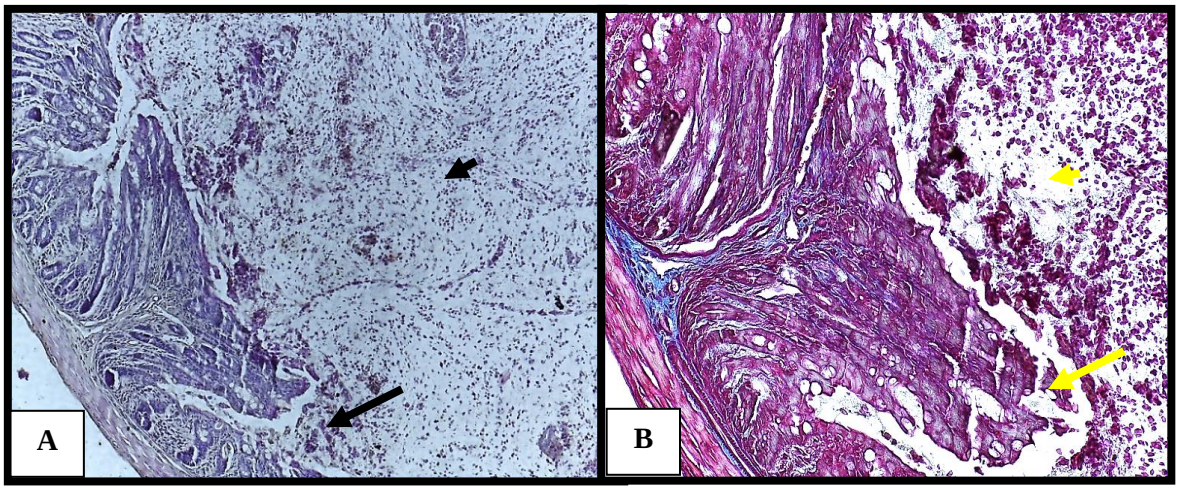

Fig. (7) Colon at day 7; Acute inflammatory exudate of polymorphonuclear leucocytes, RBCs, fibrin, and mucus (arrow) erupting from a small ulcer in the colonic mucosa of infected mice at day 7, This picture is compatible with pseudomembranous enterocolitis (arrowhead).(A)H and E stain 100X(B) Masson's Trichrome stain 200X.

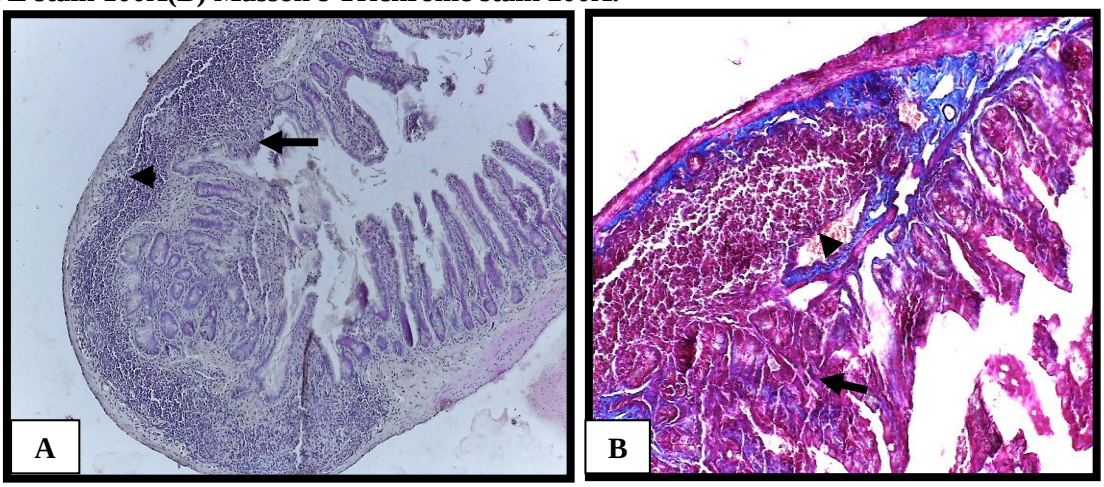

Fig. (8) Duodenum of infected mice at day 14 showing some villi which appeared eroded with sloughing of its epithelial cells (arrow) with infiltration of mononuclear (lymphoid hyperplasia) cells with blood vessels congestion (arrowhead). (A) H and E stain 100X (B) Masson's Trichrome stain 200X.

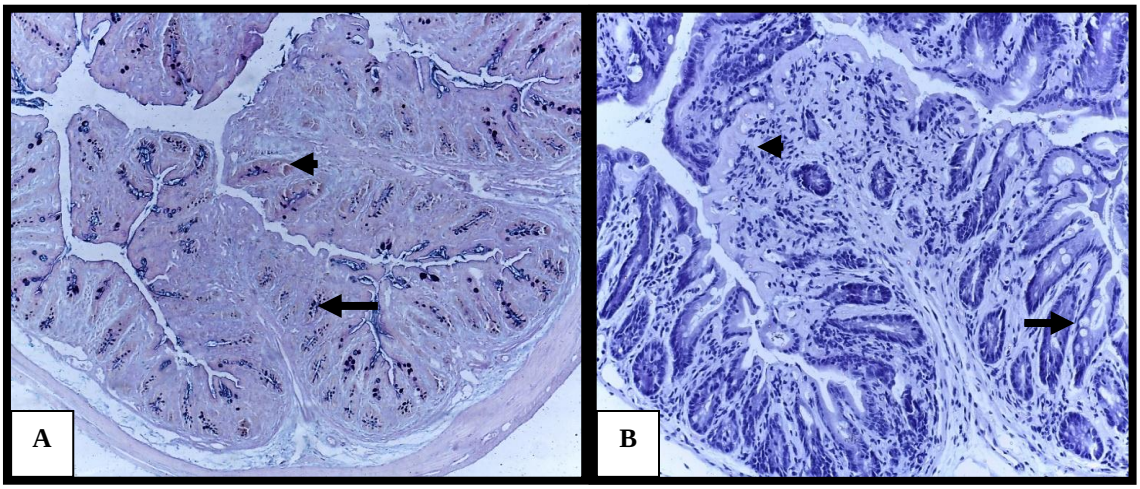

Fig. (9) Cecum from infected mice at day 14 showing few Alcian blue-positive goblet cells (depletion) at intestinal epithelial (arrow) with inflammatory cell infiltration (arrowhead). (A) Alcian blue stain 100X (B) H and E stain 200X.

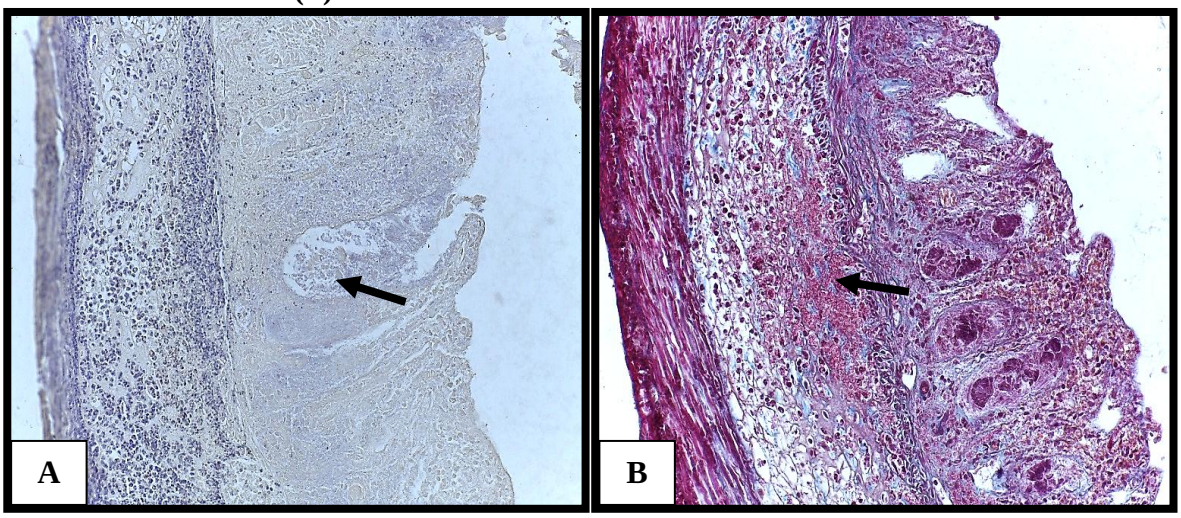

Fig. (10) Erosive changes of colonic epithelia were noticed in the infected mice at day 14 with sever hemorrhage (arrow) (A) H and E stain 100X (B) Masson's Trichrome stain 200X.

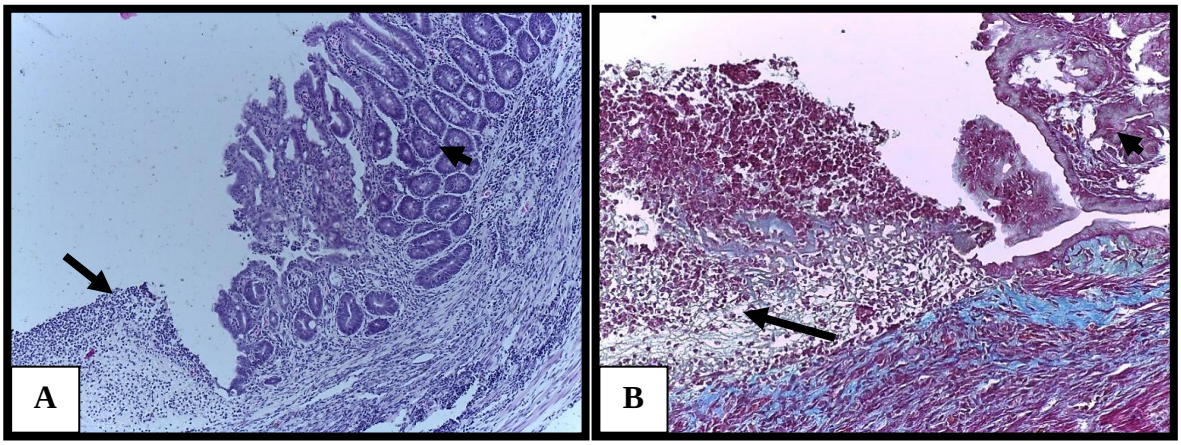

Fig. (11) Cecum of infected mice at day 21 showed epithelial layer erosive (arrow) with regenerative epithelial (arrowhead) (A) H and E stain 100X (B) Masson's Trichrome stain 200X.

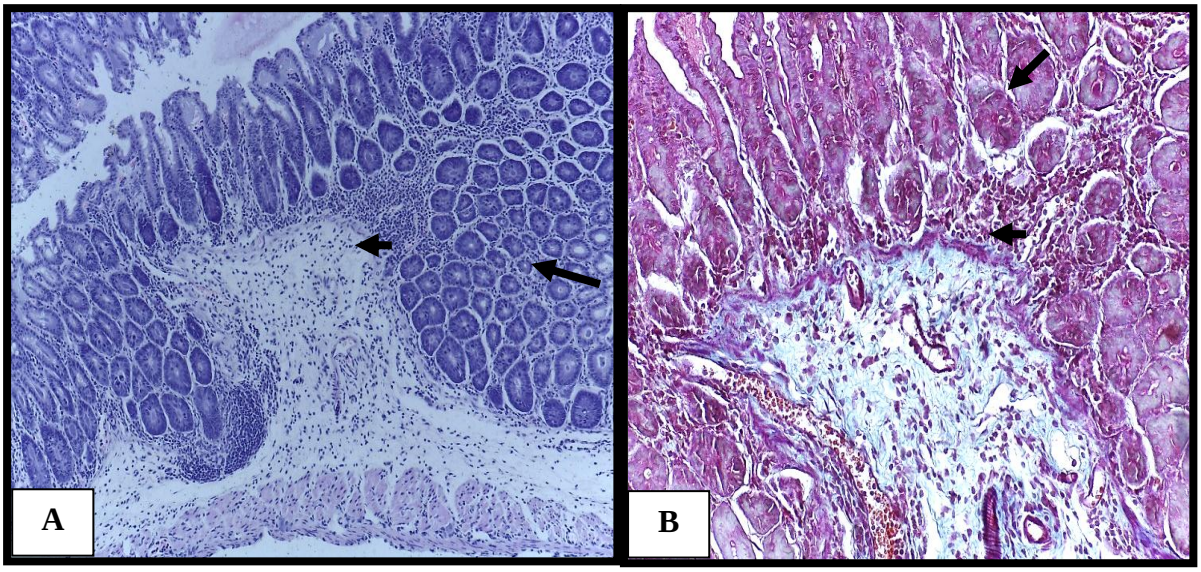

Fig. (12) Colon at day 21 of infected mice showed regenerative process of epithelial layer (arrow) with a mild inflammatory cells infiltration and submucosal thickening (arrowhead).(A) H and E stain 200X (B) Masson's Trichrome stain 400X.

Discussion

Infected mice intestine showed all stages of sloughing, erosion and complete necrosis of the villi also fusion of some villi together, necrosis of epithelial cells lining the intestinal glands, wide spread inflammation, including inflammatory cells infiltration and proliferation in lamina propria and crypt abscesses. Moreover, increased in the goblet cells as well as depletion it, were in agreement with (35, 36, 37, 38). These lesions may be attributed to the fact that the isolates of STEC O26 produced several factors which contribute to their virulence; Shiga like toxin 1, 2 and several proteins encoded in locus of enterocyte effacement pathogenicity mainly endothelial cells lining the blood vessels leading to vascular damage and haemorrhage (39, 40). These observation agreed with (41) who reported that A/E pathogens induced cellular damage characterized by cellular necrosis, disruption of the epithelium and occasionally bleeding, in addition it may be due to occlusion of vascular sinusoids by thrombus and inflammatory cells. (39), mentioned that after STEC colonization in intestine, the stx enter the circulation and transferred to blood vessels endothelial cells and that lead to damage with fibrin deposition, stx also causing direct damage to other tissues. (41), mention that the vascular damage by stx may allow to lipopolysacharride and other inflammatory mediators to gain access for circulation and initiating inflammation (42). In addition (43) reported that intravenous injection of (Stx1) in rabbits causes lesions in the intestine (cecum). Also (44), found that severe lesions in the duodenum, manifested by large amount of fibrin necrotic debris, sloughed epithelial cells. Goblet cells hyperplasia as intestinal epithelial response to the process of inflammation induced by STEC O26 infection, goblet cell hyperplasia and hypertrophy act as a defense trial from the body against the microorganism invasion and its toxin production. That the overproduction of mucins are commonly associated with inflammation of intestine infected with bacterial also it play role to prevent the bacterial attachment (45, 46**,** 47). goblet cells hyperplasia shown in other case of injury (48, 49, 50), which leading scientists to propose that this response make goblet cell secreted mucin to form a viscous gel like that catch microorganisms or irritants to the epithelia that prevents their access to

the epithelium (51). At the late stage of infection goblet cell depleted may be due to loss of crypts and surface epithelium; these finding were in agreement with (52, 53). These changes are happened with the intestinal inflammation which seen in humans with EPEC (8). This study showed that a single intragastric inoculation of STEC O26 could induce duodenitis, typhlitis and colitis in mice. This may be important ecologically since some foods or drinks such as cow-related products are exposed to contamination with STEC O26. Our results suggested that ingestion of foods or drinks contaminated with an increased number of STEC O26 may produce harmful effects on intestine.

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