Effects of sorbitol fermenting STEC (O157: H-) in experimentally infected mice on blood urea and histology of colon and kidney

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Abstract

Shiga toxin producing *E. coli* (STEC) are an important zoonotic disease and a food contaminated pathogens of human prompting serious illness in gastrointestinal tract such as hemorrhagic colitis and intense renal failure. This study was intended to research the influence of tentatively infected mice with STEC O157: H- on blood plasma urea and histopathological changes in colon and kidney. STEC Strain, which has been used in this study was (O157: H- positive to both stx1 and stx2) by PCR analysis, Sixty male mice ranged from 8 to 12 weeks old, were partitioned haphazardly into two groups (n=30), first one (infected group) were orally inoculated with (10^{10} CFU/ml in PBS), while the second gathering (control group) was gotten PBS orally. Three, seven and fourteen days post infection, blood were collected from 10 animal to each group by cardiac puncture to measure the blood plasma urea, then the colon and kidney were removed for histopathological analysis to recognize the influence of contamination on these organs. The results demonstrated huge expand ($P<0.05$) in infected at contrasted with a control set at all experimental period meanwhile infected group showed a significantly increased ($P<0.05$) in urea level at day 7 and 14 in contrast with day 3 of a test. The histopathological investigation includes; colon and kidney illustrated mild diffuse inflammation which includes expanded inflammatory cells in tissue intraepithelial and goblet cells hyperplasia. Renal tubules showed contracting lumen with epithelial cell extensive injured, swelling and generalized necrotic characterized by acute tubular necrosis. The study concluded that tentatively contaminated mice with isolated strain (O157: H-) from children showed elevated of blood plasma significantly urea after infection, with sever pathological changes in colon and kidney at all time of investigation portrayed by sever colitis and intense kidney necrosis.

Key words: Sorbitol fermenting STEC (O157: H-), blood urea, histology of colon and kidney, mice
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تأثير الاشيريشية القولونية الفارزة لسموم (stx) والمخمرة للسوربيتول النمط المصلي (O157: H−) للإصابة التجريبية في الفئران على مستوى اليوريا في الدم وعلى التغيرات النسيجية للقولون والكلى

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الخلاصة

الاشيريشيا القولونية الفارزة لسموم (stx) تعتبر من الجراثيم الانتقالية ومن الملوثات المهمة لطعام الانسان والتي تسبب امراضا حاده له وبخاصة في الجهاز الهضمي حيث تسبب التهاب القولون النزفي وكذلك تسبب الفشل الكلوي الحاد. تهدف هذه الدراسة الى التحري عن تأثير الاصابة المستحدثة تجريبيا في الفئران بواسطة النمط المصلي (O157: H−) المعزولة من الاطفال المصابين بالإسهال والتي كانت موجبه لـ (stx1 و stx2) عند فحصها بالـ (PCR) على مستوى اليوريا في الدم والتغيرات المرضية النسجية للقولون والكلى. تم استخدام ستين فأرا ذكرا بعمر 8−12 تم تقسيمها بشكل عشوائي الى مجموعتين: المجموعة الاولى (مجموعة الاصابة) استحدثت فيها الاصابة بالعترة

المصلية (O157:H–) عن طريق الفم وبتركيز (10^{10} CFU/ml 10) في حين ان المجموعة الثانية (مجموعة السيطرة) تم تجريعها بمحلول الملح الفسلجي 1 مل وبعد 3، 7 و14 يوما من الاصابة (10 فئران من كل مجموعة ولكل فترة) حيث تم جمع الدم من القلب مباشرة لقياس كمية اليوريا في الدم وبعد ذلك تم التضحية بها وتم استخراج القولون والكلى لإكمال الدراسة المرضية النسجية. اظهرت النتائج زيادة معنوية ($P<0.05$) في مستوى اليوريا للحيوانات في المجموعة المصابة مقارنة مع مجموعة السيطرة في كل مراحل التجربة كما كان هنالك ارتفاعا معنويا ($P<0.05$) في اليوم السابع والرابع عشر مقارنة مع اليوم الثالث في مجموعة الاصابة اما التغيرات النسجية المرضية للقولون فقد اظهر القولون مستويات مختلفة من الالتهاب تميزت بارتفاع نسبة الخلايا الالتهابية مع فرط عدد وحجم الخلايا الكأسية الفارزة للمخاط وكذلك زيادة افرازاتها اما الكلى اظهرت تضييق في قطر النبيبات الكلوية مع تفجي خلوي للبطانة الخلوية للنبيبات ومع تقدم الاصابة ازداد النخر الحاد والتدمر في الخلايا البطانية للنبيبات. استنتجت الدراسة ان الاصابة قد سببت ارتفاعا في مستوى اليوريا في الدم وتدمير حاد في نسيجي القولون والكلى في الفئران المصابة تجريبا بالعترة (O157:H–) كل مراحل التجربة.

الكلمات المفتاحية: السوربيتول النمط المصلي (O157: H–)، اليوريا في الدم، التغيرات النسيجية للقولون والكلى، الفئران.

Introduction

Escherichia coli strains belong to Enterohemorrhagic pathotype are Shiga toxin producing *Escherichia coli* ((STEC)), it is a zoonotic pathogen and fit for creating extreme gastrointestinal ailment in human that can bring about various illness, including Hemorrhagic colitis (HC) and Hemorrhagic uremic disorder (HUS) (1), this pathotype has risen in the most recent 30 years as a standout amongst the most vital reasons for intestinal disease (2, 3). STEC are not invasive strain, so bacteremia was unusual, but there result on body through causes a ribosome inactivating by toxin called Shiga-like toxins (Stx), which are responsible for organs damage (4, 5), The influence of Stx is intervened by restricting stx to the receptor called globotriaosyl ceramide (Gb3) (6), which inhibits protein synthesis then cell killed (7, 8). 2 types of Stx are created by STEC were Stx-1 and Stx-2.; Stx-1 contrasts in one and only one amino corrosive from the Shiga poison made by S. dysenteriae, and Stx-2 is homologous with Stx-1 in around 60% (9). The most pervasive group of STEC are O157:H7 and O157:H7-, E. coli O157:H7 was initially reported as a pathogenic microbes in flare-up of gastrointestinal disease in 1982 in the USA (10). Meanwhile, O157: H- strains were first identified in 1988 in Germany (11). The O157: H– strains is a sorbitol-fermenting (b-glucuronidase-positive) as opposed to O157:H7 is a non-sorbitol-fermenting (b-glucuronidase-negative), so O157: H- cannot isolate on a commercial agar or media which used to isolation STEC O157 (Sorbitol MacConkey agar), O157: H- have now developed as most basic causative operator of illness, including life debilitating HC and HUS (12, 13, 14, 15) Some evidence suggests that O157:H- is more oftentimes connected with STEC disease than O157:H7 (16, 17). O157: H-demonstrates expanded adherence to colonic epithelial cells and bringing about systemic provocative response inclining for high hazard for HUS (5). O157: H- causes several outbreaks in worldwide, in Germany, United Kingdom, Hungary and Finland (15, 18, 19, 20) similarly in Czech, Austria, Ireland, Belgium and Norway (21, 22, 23, 24, 25, 26). The seclusion of O157: H- was not reported outside Europe until 2002, when detected from patient with HUS in Australia (27). HUS have extreme signs described by thrombocytopenia, hemolytic anemia, and intense renal serious damage. HUS related to STEC causes intense kidney

damage in Children matured five years who are more probable vulnerable to create inconveniences need to hospitalization and dialysis for kidney to keep it work (28). STEC were in charge of 70–90% of HUS due to its endemic nature in these nations (22, 29, 30). In (1985), Karmali and partners were first clarified the relationship amongst Stx and diarrheal identified with E. coli disease and the idiopathic HUS of kids and earliest stages (31). Kidney was the preferential target in extra-intestinal for Stx, because kidney renal epithelial and endothelial have abundant expression and have a high level of Gb3, (8, 32). HC by STEC produces diarrhea, or watery diarrhea involving speckled or grossly bloody stools, and involves including spotted stools with blood, and includes feverless cramping, stomach torment and regurgitation (33). Several complications may arise from HC by STEC range from enteropathy to gangrene with peritonitis and sepsis, to rectal prolapse, coma, neuronal signs as hemiplegia also pancreatitis and seizures were recorded (33, 34).

Materials and Methods

- **Bacteria preparation and harvesting:** *E. coli* Strain O157: H- produces both stx1 and stx2, the bacterial strain (identified by PCR for serotyping and stx gene) collected from characterized stock, which has previously being maintained for research in the laboratory Department of Pathology, College of Medicine-University of Al-Muthanna, Iraq. Some bacteria were cultured into a glass petri dish with MacConkey agar by the utilization of a sterile loop. A colony from the harvested was cultured on an EMB agar to confirm that the colony was *Escherichia coli*. Tryptic soy broth (100 ml) was used to calculate bacteria count according to (35).
- **Mice:** Sixty germ-free albino male mice between 8 - 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 (6mg/ml) according to (36), mice was starved from 18 to 24 h from food at that point partitioned into two groups (n=30) :(Group 1) control animals receiving 1 ml from PBS, and (Group 2) experimental animals receiving STEC O157: H- (10^{10} CFU/ml in PBS) as infective dose according to (37). At the days 3,7 and 14, ten mice of every group were murdered by cervical disengagement after collection of blood to determine urea in plasma were done by using commercial kits (Urea-B kit, SPINREACT, SPAIN), The kidney and colon were aseptically collected for histological investigation.
- **Histological examinations of tissues**: Kidney and colon of control and STEC-infected mice were washed with PBS, fixed Tissue specimens in 10% neutral formalin-buffered solution, dehydrated and inserted with liquor and paraffin individually. Sections (4 mm thick) then colored with schedule stain H and E (haematoxylin and eosin), Alcian blue stain at 2.5 ph., furthermore, PAS stains were utilized according to (38) for light microscopy viewing.
- **Statistical Analysis**: *t*- test and One-Way ANOVA by using Statistical Package for Social Science (SPSS) system to analyze the information of our study (62).

Result

- **Serum urea:** The results of serum urea expressed as (mg/dl) indicated significant expansion ($P<0.05$) in tainted group contrasted with control at all test period meanwhile infected group showed a significant results ($P<0.05$) in urea level at day 7 and 14 compared to day 3 experiment. Table (1).

Table (1) Urea serum concentration (mg/dl) in infected and control groups

Groups / Time	Infected group	Control group
Day 3	58.46±2.73 A c	27.95±1.94 B a
Day 7	75.35±5.31 A b	29.59±1.24 B a
Day 14	102.72±7.11 A a	30.18±0.93 B a

- **Values are expressed as mean ± SE**
- **n= 10/group,**
- **Capital letters denote significant difference (P<0.05) within a row, Small letters denote significant differences (P<0.05) within a column.**

- **Histopathological changes**

- Colon: 3-day post infection period (P.I.P.): increase in inflammatory cells infiltrate, goblet cells hyperplasia also hypertrophy and bulging of mucus droplets oriented towards the lumen clearly seen as positive to Alcian blue stain, thickening of the submucosa edema and lumen filled with inflammatory cells. Fig. (1 and 2). 7-day (P.I.P.): a short, thin, and irregular shape with necrosis and ulceration with inflammatory cell. Fig. (3). 14-day (P.I.P.): the lumen become filled with inflammatory exudate of and polymorphonuclear leucocytes, RBCs meanwhile fibrin and mucus erupting from a ulcerated space. Fig. (4).
- Kidney: 3-day (P.I.P.): acute cell swelling of tubular lining epithelia causing diminished lumen diameter, intertubular inflammatory cells infiltrate with extravasated area. Fig. (5). 7-day (P.I.P.): deposition of irregular droplets protein particle of different size within the convoluted tubules as indicated by PAS stain (PAS positive). PAS positive material appears in the cytoplasm and brush borders of proximal tubules. Fig. (6). 14 day (P.I.P.): sever necrosis with inflammatory cell infiltration into tubular sit. Fig. (7).

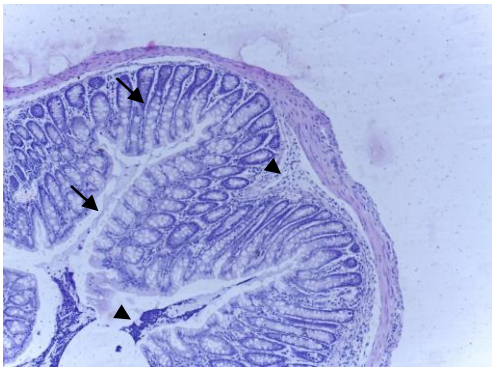

Fig. (1) colon at day 3 showed goblet cells hyperplasia with increase of mucin droplets oriented towards the lumen (arrow) edematous submucosa and infiltration of inflammatory cells in submucosa and colon lumen (arrow head) H & E stain 200x

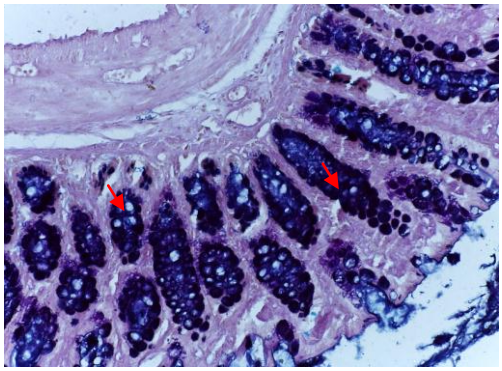

Fig. (2) colon at day 3 with Alcian blue staining positive material indicative to mucin and revealed increased numbers of goblet and hyper secretion (arrow). Alcian blue stain 200x

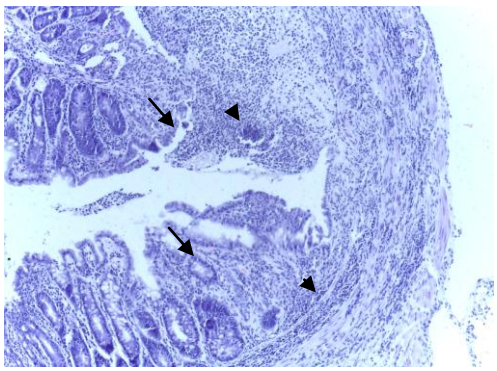

Fig. (3) Colon at day 7 shows villi fused together with complete necrosis of lining epithelial cells with ulceration (arrow) and with increased in the numbers of intestinal inflame cells within all colon layer (arrow head). H&E stain 200X

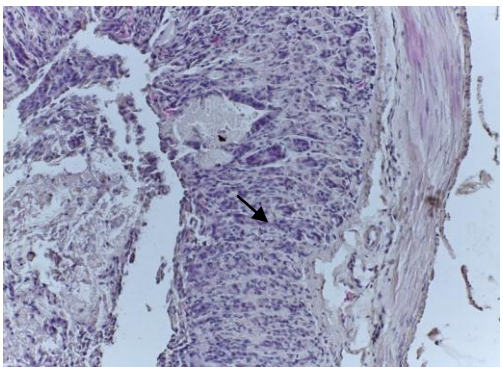

Fig. (4) Colon at day 14; inflammatory exudate of polymorphonuclear leucocytes, RBCs, fibrin, and mucus were erupting from ulcer in the colonic mucosa (arrow). H and E stain 200X.

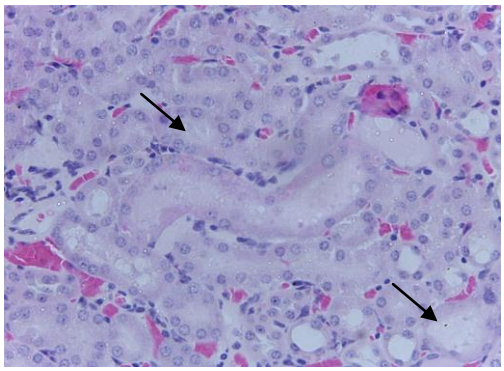

Fig. (5) section in the kidney at day 3 post infection showed acute cellular swelling of epithelial cells (arrow) with mild hemorrhage of interstitial tubules (arrow head). H and E stain 200X

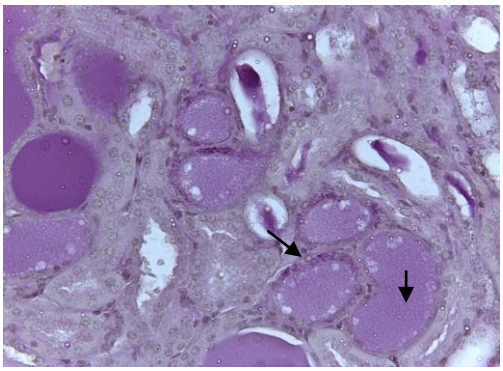

Fig. (6) kidney of infected mice at day 7 post infection showed PAS- positive, irregular sized protein droplets in tubules and inside the epithelial cells (arrow).with hyaline degeneration (arrow head). Periodic acid-Schiff (PAS) stain 400X

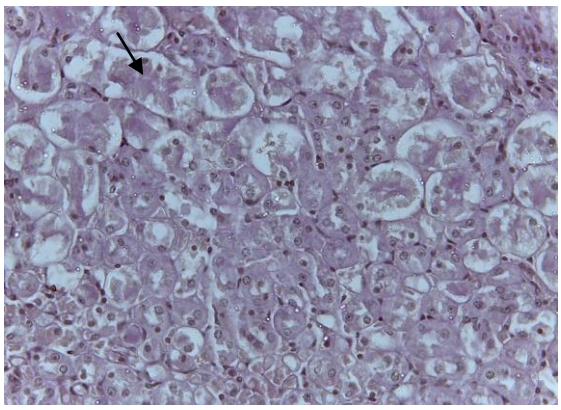

Fig. (7) Histopathological section in kidney at day 14 post infection showed acute tubular necrosis of epithelial cells (arrow) with inflammatory cells. Periodic acid-Schiff (PAS) stain 200X

Discussion

The study showed elevated blood urea in the infected group as compared to control group, is due to permanent renal failure and tissue damage. The permanent renal failure occur due to STEC infection was established (39, 40, 41). A similar result was conducted by (42), when they tainted mice with E. coli O157:H7 and watched confirmation of increased blood urea. Intense renal damage can be impelled by intravenous infusion of Stx (43), also animals when infected with STEC possess Stx2 causing evaluated blood urea at 72 h post bacterial infection causing renal damage (44). A similar to that result was observed in humans by (43). The lesions in colon may be attributed to the fact that the O157:H- produces several factors which contribute to their virulence; Shiga like toxin 1, 2 and several proteins encoded in a locus of enterocyte effacement are mainly endothelial cells lining the blood vessels leading to vascular damage and haemorrhage (45, 46). After colonization of STEC in digestive tract, the stx enter the circulatory vessels and leading to harmful effect with fibrin formation, stx additionally creating direct harm to different tissues (45). The vascular damage by stx may permit to LPS and other virulent and inflammatory mediators to contact for circulation and starting inflammation (47, 48). In addition (49) reported that vein dose of (Stx1) in rabbits effects sever lesions in the intestine. Goblet cells hyperplasia as intestinal epithelial response to the process of inflammation induced by STEC infection, goblet cell hyperplasia and hypertrophy go about as a resistance trial from the body against microorganism attack and its poison generation (50). The overproduction of mucins are regularly connected with enteritis because of bacterial contamination, it assumes a part averting bacterial connection (51, 52). The histopathological finding of kidney was in agreement with other study done on mice and kids with STEC contamination (44, 53). After creation of stx in the digestive tract, poisons spread to blood dissemination through intestinal neutrophil and epithelial (54). Other study (55), reported that there is expanded in levels of the inactivated stx in the kidney most likely reflected by freedom of the denatured protein tubular harmfully effect was identified in mice after exploratory vaccination with stx1 intraperitoneally (56, 57), what's more, kidney glomeruli and intense tubular necrosis reported in creature with STEC (58). Kidney damage most likely because of a high basal level of Gb3 (59). Histological changes in the kidney was because of the stx2 creating by STEC and that lead to tubular necrosis (44). In human HUS 20% of patients create intense renal damage and in end-stage kidney stopped working (60, 61). **The study concluded**; That the isolated strain (O157:H-) from children which was positive to (Stx1 and Stx2) experimentally infected mice, cause significant increase of blood urea after 7 and 14 day from infection, and lead to important pathological changes in; colon and kidney at all period of experiment characterized by sever colitis and intense renal necrosis.

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