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Purification of staphylococcal enterotoxin B from local isolates and evaluation of its effect on stomach and small intestine tissues

Khadija. I. Hamad*, Ahmed. S. Farhan

Department of Biology, College of Science, University of Anbar, Ramadi, Iraq
*E-mail:kha21s1001@uoanbar.edu.iq

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ABSTRACT

This study aimed to extract staphylococcal enterotoxin b (SEB) from various clinical samples (urine, wound, blood, and eye swab) in Iraq. SEB was further purified through ion exchange chromatography as a primary step followed by gel chromatography. Acids were altered through ammonium sulfate precipitation because ammonium sulfate completely meets requirements as a salt, including having a high dissolution rate in water, component ions that are safe for use with proteins, and low cost and being a low-heat solution and commercially available. Absorption peak (15–38) fractions that represent SEB with high activity were detected. After the extracted SEB was administered to laboratory animals (rats), strong pathogenicity signs, including lethargy, withdrawal, decline in activity, and change in the cornea, were observed, which lead to the death of the animals only 48 h after the second dose. The protein concentration was 0.2 mg/ml. We conclude that SEB is a highly potent super antigen that causes a range of histopathological changes in some body organs, and the severity of the effects depends on SEB dose, period, and experimental animals. In addition, SEB causes death in rare cases

Introduction

The main human pathogen *Staphylococcus aureus* produces a toxin known as staphylococcal enterotoxin B (SEB), which is a potent super antigenic toxin and is regarded as a bioweapon. However, a genetically characterized mutant that demonstrates the role of SEB in pathogenesis following a *S. aureus* infection has been identified in a clinically relevant strain of *S. aureus*. The pathophysiology of *S. aureus* infections is influenced by SEB, and this effect indicates the importance of targeting SEB in multimodal antistaphylococcal drug development. Furthermore, SEB leads to the lethal aggravation of community-associated methicillin-resistant *S. aureus* infections[1]. SEB is one of the main virulence factors of *S. aureus*, but targeted medications are currently insufficient. By binding with MHC-II on antigen-presenting cells, SEB activates immune cells, inducing the production of pro-inflammatory cytokines. The overactivation of immune cells can be prevented by inhibiting interactions between SEB and MHC-II[2].

*Corresponding author at : B Department of Biology, College of Science, University of Anbar, Ramadi, Iraq
ORCID:https:// https://orcid.org/0000-0000-0000-0000 ,
Tel: +964 7000000000
Email: kha21s1001@uoanbar.edu.iq

Through interactions with intestinal epithelial cells and vagus nerves, which connects the central nervous system to various organs, staphylococcal enterotoxins (SE) induce emesis (vomiting) in humans and monkeys. However, the exact mechanism of action is still unknown. *S. aureus* secretes a related emetic enterotoxin called staphylococcal enterotoxin A, which attaches to afferent neurons and submucosal mast cells after quickly crossing the intestinal epithelium through transcytosis. The enterotoxin promotes the degranulation of mast cells and the release of the neurotransmitter serotonin. These effects probably trigger afferent vagus nerves and activate the brainstem-based vomiting center[3].

Physiological Effects:

: SEB has a distinct mechanism of action and causes various symptoms, including fever, dyspnea, lethargy, systemic inflammation, vascular leakage, and hypotension because of its super antigenicity and capability to hyperactivate T cells. The lethal toxic shock syndrome caused by SEB is characterized by

extensive intravascular coagulation, considerable vascular leakage, and multiple-organ failure leading to hypovolemic shock. In addition to inducing immediate local inflammation in the gastrointestinal system, SEB causes severe diarrhea, vomiting, leukocyte infiltration, and inflammatory lesions of the intestines. The lungs of rhesus monkeys suffered from severe pulmonary edema, lesions, hyaline membrane formation that clogged the alveoli, fibrin deposition, and leukocyte infiltration and eventually died after exposure to SEB[4].

Materials and Methods

Culture of Toxin-Producing *S. aureus* Isolates

An *S. aureus* strain was cultured for 18 h with aeration in a pH 6.5 medium containing 1% N-Z-amine A, 1 g of powdered protein hydrolysate, thiamin, and 0.001% nicotinic acid. Protein content was determined using the Bradford technique, which involves performing centrifugation on a chilled culture for the removal of bulk cells and measuring UV absorbance at 280 nm. Protein activity was measured in laboratory animals (rats) exposed to SEB, and their organs were harvested for histological study. The effects of SEB on the stomach and small intestine, morphological changes in activity, and weight loss were examined[5].

Ammonium Sulfate Precipitation and Dialysis

To precipitate ammonium sulfate, the toxin's crude extract was progressively mixed with ammonium sulfate on ice at varying saturation ratios. The mixture was then centrifuged for 20 min at 4 °C and 6000 rpm. The supernatant was discarded, and the precipitate in each concentration was dissolved in suitable amounts of phosphate buffer solution, dialyzed in a dialysis tube at 3500 Mw cutoff against potassium phosphate buffer (pH 7) for 24 h at a low temperature (4 °C), and protein concentration was measured[6].

Purification by Ion Exchange Chromatography

Diethyl-amino-ethyl cellulose (DEAE-cellulose) was prepared. A DEAE-cellulose resin (20 g) was suspended in 1 l of distilled water, and the resulting beads were allowed to stabilize and rinsed repeatedly with distilled water. Under discharge, the suspension was filtered with Whatman No. 1 filter paper through a Buchner funnel. The resin was then re-suspended in sodium chloride (0.25 M) and sodium hydroxide (0.25 M) solutions. The suspension was filtered again and rinsed with hydrochloric acid multiple times (HCl; 0.25M) and subsequent with distilled water before equilibration with Tris-HCl buffer (pH 8.5). The resin was then packed into column (3 cm × 20 cm). An enzyme solution was then added to a DEAE-cellulose column. After the bound proteins were gradually eluted using increasing concentrations of sodium chloride (0.1–1 M), the column was cleaned with an equivalent amount of the same buffer. Using a UV-visible spectrophotometer, the absorbance of each fraction was measured at 280 nm with a 3 ml flow rate across the column[7].

Purification by Gel Filtration Chromatography

Sephadex G-150 was used in accordance with Pharmacia Fine Chemicals Company recommendations. A suitable quantity of Sephadex G-150 was suspended in potassium phosphate buffer (pH 7), heated at 90 °C for 5 h, degassed, loaded in a glass column (2 cm × 40 cm), and adjusted with the same phosphate buffer for the expansion of the beads. After undergoing ion exchange and cleaning, the purified toxin was applied to the matrix. The elution process was then carried out at a flow rate of 3 ml/fraction with the same buffer for equilibration. Lastly, the absorbance of each fraction was measured at 280 nm[8].

Results and Discussions

Extraction of Staphylococcal Enterotoxin B (SEB)

The *S. aureus* isolate that produced SEB was cultivated in N-Z-amine A medium (pH 6.8) and incubated for 24 h at 30 °C in a shaking incubator. The culture broth was centrifuged under refrigeration, and the supernatant was discarded, and the pellet. The SEB exotoxin was produced in the medium and utilized as a crude toxin extract. Protein concentration was 0.41 mg/ml with a Bradford protein assay at 280 nm.

Ammonium sulfate precipitation

Ammonium sulfate precipitation is the preferred technique. Almost all proteins precipitated from the solutions when salt concentrations were high enough. Protein surfaces frequently feature hydrophobic patches, which are encircled by organized water molecules in a solution. The hydrophobic areas of the protein surfaces are gradually exposed when salt is introduced to protein solutions because water is drawn in to solvate the ions of

the dissociated salt. These patches eventually begin to interact, causing aggregation and the precipitation of proteins in the solutions. Ammonium sulfate completely meets the requirements as a salt, such as having a high dissolution rate in water, component ions safe for use with proteins, and low cost and being a low-heat solution and commercially available. Proteins in a solution precipitates after the addition of ammonium sulfate, and proteins are recovered through centrifugation, resuspended in a low amount of water or buffer, and then remove any remaining ammonium sulfate through dialysis[9]. The protein concentration was 0.5 mg/ml.

Ion Exchange Chromatography

Adsorption is represented stoichiometrically in most well-known adsorption models, which use the law of mass action as the basis. Despite the usefulness of these models in model-based research, the stoichiometric representation of adsorption process is not adequate for the description of long-range electrostatic interactions in ion exchange chromatography, restricting the application and mechanistic expansion of these models[10]. The DEAE-cellulose column was loaded with the crude toxin extract, any positively and uncharged proteins from the sample were removed through washing, and the sample was equilibrated with an equivalent volume of Tris-HCl buffer (pH 8.7). A high pH was used because the isoelectric point of the SEB toxin was 8.6. When pH > Pi, proteins carry negative charges on their surfaces and attach to positively charged anion exchangers. The bound negatively charged proteins were subsequently eluted using gradient concentrations of sodium chloride (0.1–1 M)[11]. Fig. 1 shows one protein peak in the wash elution and one peak in the gradient elution. The eluted proteins (fractions 27–47) contained most of the SEB activity, which was observed after the application of the toxin on animals and regarded as pathogenicity signals generated by SEB. The protein concentration was 0.3 mg/ml.

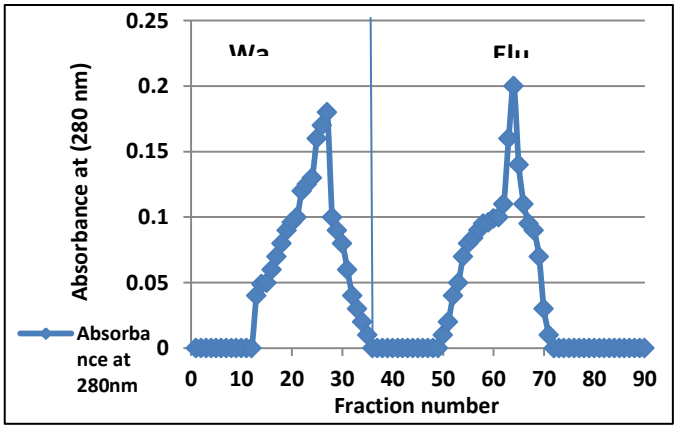

Figure 1: Wash and elution profiles of the purified *S. aureus* enterotoxin b after ion exchange chromatography performed using a DEAE-cellulose column (3 × 20) cm. The column was calibrated with Tris-HCl buffer (pH 8.5), the flow rate was 36 ml/h, and the fraction was 3 ml.

Purification by Gel Filtration Chromatography

Gel filtration, also known as exclusion chromatography, is a technique that divides particles or molecules according to their hydrodynamic radius on the basis of how differently they can pierce the stationary phase's gel pores[12].

Fig. 2 demonstrates fractions with one absorption peak (15–38) that represents SEB with the highest activity. After SEB was applied on laboratory animals (rats), strong pathogenicity signs were observed, including lethargy, withdrawal, a clear decline in activity in response to the effect of the toxin, and a change in the cornea of the eye, which lead to the death of the first animal after only 48 h after the second dose. The protein concentration was 0.2 mg/ml.

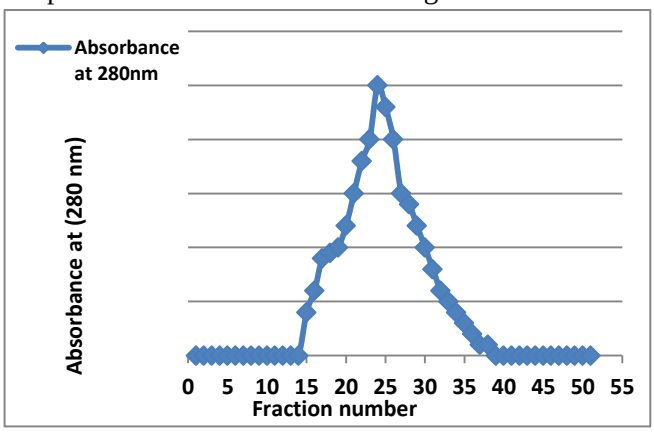

Figure 2: Gel filtration chromatography on Sephadex g-150 for SEB purified from *S. aureus* isolate (2 cm × 40 cm). The

column was calibrated with potassium phosphate buffer (pH 7), the flow rate was 36 ml/h, and the fraction was 3 ml.

Table (1) Purification steps

Purification step	Volume (ml)	Protein concentration (mg/ml)
Crude enzyme	100	0.41
Ammonium sulphate precipitation 50%	15	0.5
DEAE-cellulose	21	0.3
Sephadex- G100	18	0.2

Histopathological Changes in the Stomach & Intestine

The histopathological figures of small intestine showed mild enteritis characterized by the mild thickening of mucosal villi. This effect is associated with the infiltration of mononuclear leukocytes and hyperplasia of the lining cells of intestinal glands with luminal debris (Fig.3). While the figure of gastric mucosa and gastric glands were similar those in the control group (Fig. 4). Marked epithelial sloughing and hyperplasia of mucous-secreting goblet cells within intestinal glands (Fig. 5) and marked hyperplasia of the mucous-secreting goblet cells of intestinal glands with luminal necrotic tissue debris (Fig. 6) were observed. Pathophysiological changes associated with intestinal SEB exposure were observed because SEB is absorbed by the intestinal epithelium and acts as a super antigen through its ability to cross-link MHC class II molecules on antigen-presenting cells with the Vβ chains of T cell receptors on T cells in the lamina propria. This polyclonal activation of T lymphocytes increases the production of cytokines including interferon-g IFN-γ, IL-2, and tumor necrosis factor-a, which are linked to toxin-induced symptoms, including food poisoning and toxic shock syndrome, in humans and other species[13].

Changes in villus morphology (thickening of mucosal villi) may affect the epithelium's ability to absorb nutrients and selectively transport electrolytes and increase intestinal permeability, enabling SEB to enter the lamina propria and the systemic circulation and causing systemic inflammation. SEB stimulates cell recruitment and the synthesis of pro-inflammatory cytokines, resulting in mild gut-associated lymphoid tissue activation. Immune activation reduces the expression of junctional proteins, such as zonula occludens-1 and catenin, which is associated with increased luminal water content and mucosal permeability. SEB lowers mucosal defensin expression and the abundance of sodium–glucose transport proteins, such as SGLT1, in the villous apex[14]. SEs exhibit emetic action but that are not emetic in a primate model or are yet to be evaluated[15]. This finding agrees with our finding, which showed that no emetic activity occurred during the dosing of animals. Leukocyte migration via the endothelium and the underlying basal membrane into the mucosa and submucosa in inflammatory bowel disease have not been comprehensively explored. Activated leukocytes migrate to the margins of endothelial cells and permeate them through amoeboid-like movements. The degree of leakage in endothelial cell connections in intestinal postcapillary venules is higher than that in the intestinal epithelium. Neutrophils undergo this process in vitro in 90 s[16].

Severe and often fatal inflammatory intestinal disease may occur if enteric exposure to a toxin is prolonged or host defenses are impaired. SEB decreased the expression levels of α-defensin cryptdin 4 and β-defensin 1 in the rat model. A decrease in cryptdin 4 expression may lead to an increase in intestinal IL-1β production because cryptdin 4 can inhibit the release of IL-1 from activated monocytes. This effect would increase the risk of inflammatory bowel illnesses and increase the susceptibility of the gut to injury due to SEB[17].

Figure (3) Section of the small intestine. Moderate thickening of intestinal villi was associated with the

severe infiltration of MNCs (asterisk), and the hyperplasia of lining cells of intestinal glands (yellow arrow) with luminal debris was observed (blue arrow). H&E stain (10×).

Figure (4) Section of the stomach: normal mucosal surface (red arrows), normal gastric mucosa (G), and muscular wall (asterisk). H&E stain.

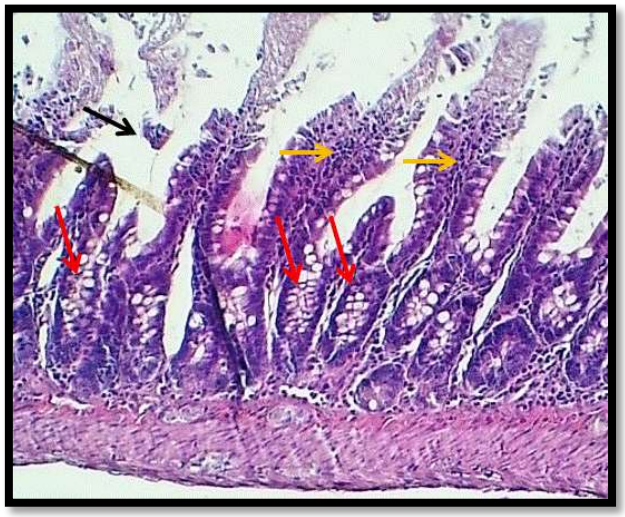

Figure (5) Section of the small intestine: moderate enteritis characterized by the thickening of mucosal villi associated with the infiltration of MNCs (yellow arrows), epithelial sloughing (black arrow), and hyperplasia of mucous-secreting goblet cells (red arrows) with intestinal glands (red arrows), normal mucosal surface (red arrow), gastric glands (G), and muscular wall (asterisk). H&E stain 10×.

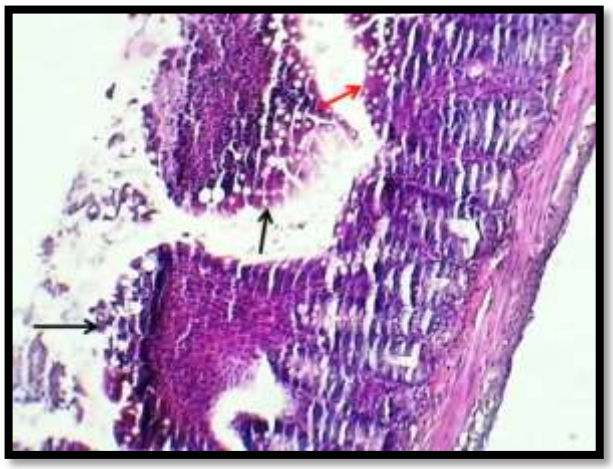

Figure (6) Section of the small intestine: severe enteritis characterized by massive damage to the intestinal villi associated with the severe infiltration of MNCs (black arrows), marked hyperplasia of mucous-secreting goblet cells (red arrows) of intestinal glands (yellow arrow) with luminal necrotic tissue debris. H&E stain 10×.

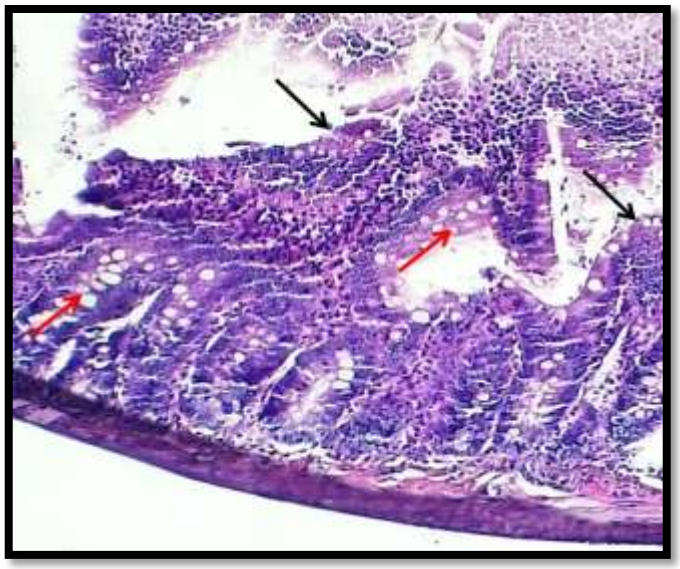

Figure (7) Section of the small intestine: severe enteritis characterized by massive damage to intestinal villi associated with the severe infiltration of MNCs (black arrows), marked hyperplasia of mucous secreting goblet cells (red arrows) of intestinal glands (yellow arrow) with luminal necrotic tissue debris. H&E stain 10×.

Conclusion

Compared with other established procedures, chromatography on DEAE-cellulose increased the yield

of a product, which was further purified by gel chromatography on Sephadex. The toxin extracted through this procedure showed high emetic power and purity. Large amounts of toxic substances (e.g., SEB) can be purified because chromatographic processes are easy to perform. Toxin activity examined by the injection of the toxin into laboratory animals via intraperitonial and intragastric routes appeared 24 h after procedure in the form of lethargy, withdrawal, and nausea. These symptoms gradually increased with SEB dose. The first animal died after the second dose (after 48 h). Notably, this purification procedure is considered incomplete, although it has been approved. Another study purified SEB with the same method but conducted SDS PAGE. The effects on animals are consistent with those reported by previous research. This finding corroborated the efficiency of the purification process.

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تنقية السموم المعوية للمكورات العنقودية ب من العزلات المحلية ودراسة تأثيره في نسيج المعدة والامعاء الدقيقة

خديجة. إبراهيم حمد[1]، أحمد سامي فرحان[2]

1، 2 قسم الأحياء، كلية العلوم، جامعة الأنبار، العراق
البريد الإلكتروني biokhadija1996@gmail.com1
البريد الالكتروني ahmed.sami@uoanbar.edu.iq2

الخلاصة:

هدفت هذه الدراسة إلى تنقية السموم المعوية للمكورات العنقودية ب (SEB) من العينات السريرية المعزولة من العدوى المختلفة بما في ذلك (البول، الجرح، الدم، مسحة العين) في العراق باستخدام كروماتوغرافيا التبادل الأيوني (IEX) كخطوة أولية يليها استخدام كروماتوغرافيا الجل لمزيد من التنقية. إن ترسيب كبريتات الأمونيوم المستخدم في تغيير الأحماض بسبب كبريتات الأمونيوم يفي بشكل كامل بمتطلبات الملح، والتي تشمل أن يكون عالي الذوبان في الماء، وأن تكون الأيونات المكونة له آمنة للاستخدام مع البروتينات، وأن تكون نسبة تركيزه منخفضة. محلول حراري. رخيص الثمن ومتوفر تجاريا. أظهرت النتائج أن هناك جزء واحد فقط من قمة الامتصاص (15–38) يمثل SEB ذو أعلى نشاط. بعد تطبيقه على حيوانات المختبر (الفئران) يؤدي إلى ظهور علامات مرضية قوية منها الخمول والانسحاب وتراجع واضح في النشاط استجابة لتأثير السم وتغير في قرنية العين مما يؤدي إلى موت الحيوان. الحيوان الأول بعد 48 ساعة فقط وبعد الجرعة الثانية كان تركيز البروتين 0.2 ملغم/مل. نستنتج أن SEB هو مستضد فائق عالي القدرة على إحداث مجموعة من التغيرات النسيجية المرضية في بعض أعضاء الجسم تراوحت من خفيفة إلى شديدة اعتمادا على جرعة SEB والفترة الزمنية وحيوانات التجارب، بالإضافة إلى أنSEB يسبب الوفاة في حالات نادرة.