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ORIGINAL ARTICLE

Isolation, Purification and Characterization of a Novel Exotoxin from *Staphylococcus aureus* Isolated from the Eczematous Lesion of Patient with Atopic Dermatitis

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ABSTRACT
A novel exotoxin from Staphylococcus aureus isolated from eczematous lesion of patient with atopic dermatitis was isolated, purified, and characterized in this study._These exotoxin has clotting activity (85.5) unit/ml, specific activity (2085.3658) unit/mg and total activity (1282.5) units after (347.56) degree of purification yielded (3.975)% of exotoxin resultant , and the solution of Staphylococcus aureus exotoxin show a very high purified single band protein by using polyacrylamide gel electrophoresis PAGE (7.5%), these band-in comparison with standard protein-has a molecular weight (43.315)kd.
Key words: Staphylococcus aureus exotoxin, Atopic dermatitis

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INTRODUCTION

The normal bacterial skin flora in human is composed of three major groups of Gram-positive bacteria, the coryneform bacteria, the micrococci and the staphylococci, with only a minor component of Gram negative bacilli [1]. This is chiefly because the skin is a comparatively dry habitat, with available water as the chief factor controlling growth; occlusion of skin is a potent way to increase the number of bacteria on the skin [2]. Gram negative bacilli require more available water than Gram-positive bacteria and this probably controls their population density [3]. Bacterial counts on unaffected kin are lower than on affected a topic skin [4]. The density of *S. aureus* on eczematous lesions has been show to correlate with cutaneous inflammation [5].

Chronic skin colonization with *Staphylococcus aureus* is a characteristic feature of a topic dermatitis (AD)(6), and about 60-90% of *S. aureus* strains isolated from the skin AD patients [7]. Up to 65% have been shown to produce exotoxins with superantigenic properties [2]. However, the mechanism(s) underlying the effects of this organism in the disease process are unclear.

These potent toxins bind directly without antigen-presenting cells (APC) such as macrophages or dendritic cells and to cytokine-induced HLA-DR molecules on non professional APC such as keratinocytes [8]. Over half of AD patients have *S. aureus* cultured from their skin that secrete superantigens such as enterotoxins A,B and toxic shock syndrome toxin-1 (TSST-1) and many of unclassified exotoxins [9,10].

There are many exotoxins that produce by *S. aureus* isolated from eczematous lesions of AD patients, and we investigate purification and characterization of one of these exotoxin.

MATERIALS AND METHODS

Primary screening

One isolate of *S. aureus* diagnosed by a routine techniques according to[(11)] ,were selected from twenty isolates according to highly degrees of chronicity and severity of atopic dermatitis associated with these bacteria.The antibacterial activity against four standard strains of bacteria was studied by culturing *S. aureus* with each of these bacteria [12] .Standard strain are: *E coli* NCTC 5933, *Staph aureus* NCTC 6571, *Kl. Pneumonia* ATCC 10031, and *B. subtilis* PCI 219.

Primary detection of *Staph.aureus* exotoxin

Two culture media were used to testing ability of *S. aureus* to produce exotoxin (having proteolytic activity): Casein Hydrolysate Agar CHA and Skin Milk Agar (SMA) (Oxoid). A clear zones around the *S.*

aureus colonies grew on above media indicate to ability of these bacteria to produce proteolytic enzyme exotoxin [13].

Production of Exotoxin

Estimation of biomass of bacterial growth (gm/100ml) in casein Hydrolysate Broth (CHB) (Oxoid), clotting activity for crude enzyme solution (unite/ml), protein concentration (mg/ml), and exotoxin activity (unit/mg) were carried according to [14].

Purification of *S. aureus* exotoxin

Three steps of purification were done for *S. aureus* exotoxin

1. Precipitation by Ammonium sulphate salt according to [14].
2. Membranous infiltration (dialysis technique) according to [15] and
3. Gel filtration chromatography by using sephadex G-100 (Pharmacia, Sweden) in column (23 x 2.2 cm) according to (16) evaluate the purity and estimate the molecular weight of ***S. aureus*** exotoxin were carried by using conventional polyacrylamide gel electrophoresis (LKB, mod. 2117 multipore, Sweden) and standard protein solution (BDH, Germany) to make standard curve:

Trypsin inhibitor, RNA polymerase, Bovine serum albumin, Aldolase, Catalase, Ferritin and Thyroglobulin.

The relative mobility (RM) was calculate by

$$Rm = \frac{\text{Distance of protein mobility until bands showed}}{\text{Distance of Bromophenol blue mobility until the current end}}$$

The molecular weight of ***S. aureus*** exotoxin was calculated by drew the relation between 10g. of M.W. of standard proteins and its relative mobilites.

These technique was carried according to [17].

Statistical analysis

ANOVA test was carried by using SPSS computer program ver.11.

RESULTS

S. aureus selected strain gave a diameter of inhibition zones (18,20), (7.5,8), (20,23) and (25,27.5) in 24 and 48hrs after incubation against standard strains of *E.coli, S. aureus , B.subtilis* and *K.pneumonia* respectively.

The growth of *Staph aureus* on CHA was heavy and gave 17mm of inhibition zone, while slow growth obtained on SMA with diameter 13mm of inhibition zone.

Production assay of *Staph aureus* exotoxin on CHA gave biomass (11g/100ml), and specific activity (7.21 unit/mg) while growth on SMA gave biomass (4 g/100ml) and (5 unit/mg) for specific activity. According to previous results illustrated that fermentation medium (growth and production media) was the best medium used to produce exotoxin from *Staph aureus*, so the CHA could use in all of future studies.

Table (1) illustrate results of purification steps of *Staph aureus* exotoxin.

The extracted crud exotoxin after precipitation by $(NH_4)_2SO_4$ gave specific activity (8.1)unit/mg, these activity higher than activity of crud extracted solution (6)unit/mg and (32.2)unit/mg with exotoxin recovery (resultant) (46.50%) after membranous infiltration and 2085.3658 with clotting activity reached (85.5) unit/ml and degrees of purification reached 347.56 with exotoxin recovery (resultant) (3.975%) after gel filtration chromatography.

Table (2) illustrate the relative mobility (Rm) and molecular weight of standard proteins and *S. aureus* exotoxin by using conventional polyacrylamide gel electrophoresis (PAGE) 7.5%.

The solution of *S. aureus* exotoxin show a very high purified single band protein picture(1) and from standard curve these band has a molecular weight (43.315)kd.

DISCUSSION

A technique composed of five steps were used to detection isolation purification, identification, and characterization of *Staph aureus* exotoxin, and after all these steps for techniques we reached a high purified single band protein has molecular weight (43.315)kd. These bacteria were isolated from eczematous lesion of patient with atopic dermatitis.

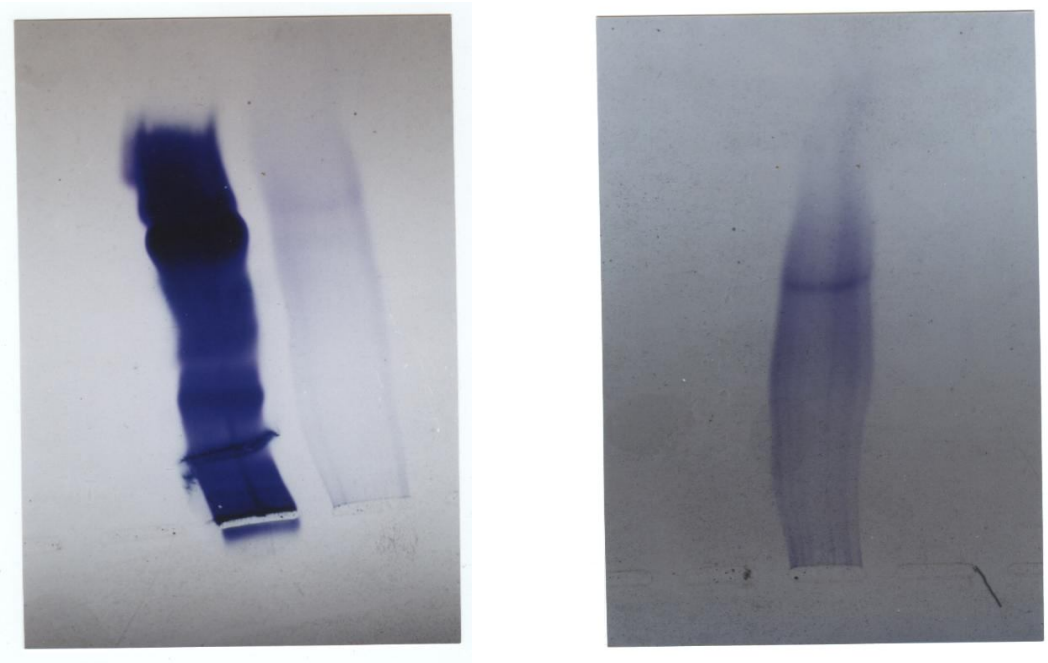

Fig 1. BANDS FROM GEL ELECTROPHORESIS .
LEFT : *STAPH AUREUS* ALL BODY ANTIGENES .
RIGTH : SINGLE PURE BAND OF *STAPH AUREUS* EXOTOXINE (SUPERANTIGENE)

Table 1: Purification steps of *Staph aureus* exotoxin

Step of purification	Volume (ml)	Clotting activity (unit/ml)	Protein concentration (mg/ml)	Specific activity (unit/mg)	Total activity (unit)	Degree of purification	Exotoxin resultant (Recovery) %
Crud exotoxin solution	500	30	5	6	15000	1	100
Precipitation by (NH4)2SO4	200	32.5	4	8.1	6500	1.35	43.33
Dialysis	200	80.5	2.5	32.2	16100	5.366	46.50
Gel filteration G-100	15	85.5	0.041	2085.3658	1282.5	347.56	3.975

Table 2: The relative mobility (Rm) and molecular weight (MW) of the standard proteins and *S. aureus* exotoxin by using conventional polyacrylamide gel electrophoresis (PAGE) 7.5%

Protein	Rm	Molecular weight Dalton
Thyroglobulin	0.092	669.000
Ferritin	0.184	440.000
Catalase	0.304	232.000
Aldolase	0.400	140.000
Bovin Serum Albumin	0.569	67.000
Chemotrypsinogen	0.676	25.000
Ribonuclease	0.705	13.700
S. aureus exotoxin	0.576	47.315.1259

Previous studies agreed and evidenced our finding suggested that a tightly correlation between *S. aureus* colonize eczematous lesion and atopic dermatitis such as Hauser, et al.[18] that found the staphylococcal enterotoxin A, B, C1, C2, C3, E, toxic shock syndrome toxin-1, and perhaps other *Staph aureus* exotoxin to be identified in the future belong to a class of proteins collectively termed superantigens and/or staphylogen may interact with immunopathogenesis of atopic dermatitis .

Abeck and Mempel, [19] confirmed our results by findings illustrated that *S. aureus* induces inflammatory reactions via a range of activities, including protein and toxin secretion. Among these are the *S. aureus* superantigens that are produced by 57-65% of isolated strains, which have been

intensively studied during the last years and have been characterized as substances with potent inflammatory and immunological effects.

Other study concluded a relationship between severity of skin lesion and sensitization to staphylococcal exotoxins in adult patients with AD, and showed that 30-60% of *S. aureus* strains isolated from AD patients are able to produce exotoxins with superantigenic properties, mostly staphylococcal enterotoxins A,B,C and D (SEA-D) and toxic shock syndrome toxin-1 (TSST-1) [20].

Exotoxin producing *S. aureus* could be isolated from the skin of patient with AD [18]. It is clear that it will not be easy to assemble supportive evidence for the fulfillment of Koch's postulates. Nevertheless this hypothesis should actively be pursued because some abnormalities found in AD may be explained by the action of superantigens. For example, superantigens presented by keratinocytes expressing MHC class II molecules may activate T cells in such a manner that they release type 2 lymphokines such as IL-4 and IL-5 but not the type 1 lymphokines IL-2 and IFN-8 [21].

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