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The Effects of Purified Staphylococcal Enterotoxin A on Gene Expression of Nuclear Factor Kappa B (NF-ĸB) in Rabbits

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ABSTRACT
This study aimed to determine the effects of Staphylococcal enterotoxin A on NF-KB gene expression in rabbits. Firstly, SEA was isolate, purified and identified from one strain of *Staphylococcus aureus* collected from urine sample, and had identified by biochemical and molecular methods. Then ,Staphylococcal enterotoxin A was purified by chromatography technique . All purified fractions of SEA was determined by ELISA kit. Finally, 5mg\ml of SEA was injected intravenously to rabbits in order to demonstrate its effects on NF-KB gene expression. At the end of this experiment, the gene expression of NF-KB increased to 9.1 in compression with control group with 1 of fold time (P = 0.045)

KEYWORDS: Staphylococcal enterotoxin A, Chromatography technique, Anion exchanger, Sephadex G-100, NF-*K*B , gene expression

INTRODUCTION
Staphylococcal enterotoxins are proteins and considered to be superantigens because it targeted T-cell leading to proliferation; cytokine releasing and cell death through the apoptosis (Otto, 2014; Zecconi, and Scali, 2013). The superantigens are reacted with major histocompatibility complex (MHC class II) on antigen- presenting cells and with the receptor of T-cell .The interaction occurs to activation specific region of the TCR -β chain, that resulting stimulation to produce more of T-cells and releasing of proinflammatory cytokines and chemokines that leading to lethal toxic shock (Chen *et al*, 2004). The primary function of superantigens is thought to weaken the host's immune system. NF-κB is nuclear factor kappa stimuli B cells; is a protein that controls DNA transcription, cell survival and cytokine production. NF-κB is present in all cell types. It formed due to cellular responses for cytokines, the stress, heavy metals, free radicals, UV irradiation, and viral or bacterial antigens (Hudson *et al*, 1995). NF-κB has a great role in regulating the immune response. Disorder control of NF-κB was leading to increase the autoimmune diseases, cancer, inflammation, septic shock and bacterial or viral infection. In addition, NF-κB has direct influences on some the genes such as P53 gene which related with DNA division (Liu *et al*, 2107; Lawrence, 2009; Oeckinghaus and Ghosh , 2009).

MATERIALS AND METHODS
Sample

This study included one determined sample indicted by their ability to produced Staphylococcal enterotoxin A. This selected sample was identified as *Staphylococcus aureus* by biochemical test (Wang *et al*, 2017; Karmakar et al, 2016) including:- Oxidase test, Catalase test, Coagulase test, Manitol salt agar , Dnase agar, Chromogenic Staph Medium, Novobiosin sensitivity and API staph system and the molecular method (RT-PCR). Also by ELISA kit was utilized for SEA detection. Bacterial DNA was extracted by Presto ^{TM} Mini gDNA bacterial kit (Geneaid).

Polymerase Chain Reaction and Real Time- PCR assay

PCR and RT-PCR were used to detect staphylococcal enterotoxins A gene. RT-PCR and PCR protocols were performed according to (Blaiotta *et al,* 2004; EI-Jakee *et al,* 2013). Table (1) showed the primer sequences of SEA and NF-KB

Bacterial Fermentation

Bacterial fermentation and enterotoxin production was carried out according to (Niskanen. and Lindroth (1976). *Staphylococcus aureus* was grown to the late stationary phase (Ghani *et al*, 2019). The supernatant was preparing for ammonium sulfate precipitation (Green and Hughes, 1955). The suspension was precipitated with ammonium sulfate at 60% saturation value.

Purification of SEs by Chromatography Technique by Anion Exchanger (DEAE-cellulose) and Gel Filtration (Sephadex G- 100)

Purification of SEs by anion-exchange chromatography according to the procedure described by (Al-Shammary *et al*, 2012)with modification by using 1M NaCl to prepare a salt gradient used for eluting the bound proteins while Sephadex G-100 was used according to (Schutte *et al*, 1997).

Human *Staphylococcus aureus* Enterotoxins A ELISA Kit Procedure

This kit was utilized to detect the presence of SEA in purified and identified samples of *S aureus* isolates. This kit is based on sandwich enzyme –linked immune –sorbent assay technology.

Table (1): primer sequence of SEA and NF-*K*B

gene	Oligonucleotide sequence (5→3)	Size products (pb)	Primer Source
SEA	CCT TTG GAA ACG GTT AAA ACG TCT GAA CCT TCC CAT CAA AAA C	127	Betley and Mekalanos (1988).
GAPDH	F-GGAGCCAAAAGGGTCATC R-CCAGTGAGTTTCCCCTTC		(Zhou *et al*, 2007)
NF-KB p65	F-AGGCAAGGAATAATGCTGTCCTG R-ATCATTCTCTAGTGTCTGGTTGG		(Le *et al*, 2018)

Administration of laboratory animals

Animals in this present study had been divided into 2 groups, control groups and treated groups.

1. **Control groups**

In control groups, three rabbits were treated with 5 ml D. W. only and were killed after 24h. This group was used to see gene expression of NFKB.

2. **Treated groups**

The rabbit in this group was injected intravenously with single dose of SEA (5µg) (Bergdoll *et al*, 1967). Animals had been killed after 24h after SEA injection. Enterotoxin A poisoning is characterized by gastroenteritis occurring 2 to 4 h after ingestion and lasting for about 24h (Kauffman and Roberts ,2006). This group was used to detect SEA effects on gene expression of NFKB.

Statistical analysis

Data has analysed using GraphPad Prism software version 8.4.3. The differences between groups were tested for significance using Mann Whitney test and Welch's test. Data has been shown as Means ± standard error of the mean (M±SEM). *p* values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Only one strain of *Staphylococcus aureus* was growing to produce SEA. 50 mg\ml was obtained from 15 liters of BHI broth. 5 µg was intravenously injected to the rabbits in order to determine the effects of SEA on NFKB gene expression.

The presented study aimed to determine the interaction and relationship between staphylococcal enterotoxin A and NF-KB activity. After staphylococcal enterotoxins A injection in rabbits, blood samples were collected to determine the difference in folding time between treated and control groups. The fold time was found (9.07) in treated group while in control group was (1)(table 2). This means that the folding time in treated group was nine times as much than the control group (*p value*=0.045, figure 1). This is evidence that there is an increasing in NF-KB activation due to SEA effects. Statistical analysis also found that the *P* value in folding time between the two groups was significant($P<0.05$).

Staphylococcal enterotoxin A (SEA) was the best characterized and was also considered as superantigens because of their ability to bind for major histocompatibility complex class II (MHC II) molecules on antigen presenting cells (APC) and stimulate large populations of T cells that share variable regions on the β chain of the T cell receptor. The result of this massive T cell activation is resulting in the stimulation of both monocytes/macrophages and cytokine. All members of staphylococcal enterotoxins are functionally related and share sequence homology. These bacterial proteins are known to be pyrogenic. Many studies were showed that only very small amounts of this toxin were needed to induce food poisoning (Nilsson *et al,* 1999). SEA has two distinctive binding sites (alpha and beta) on both sides of the peptide binding groove of class II MHC. SEA molecules must be bound to both sites for optimal activity, which allows for class II MHC crosslinking, and stable interactions with T cells. Staphylococcal enterotoxin A was known as a pyrogenic, gastroenteritis, emesis inducing and superantigenicity. Staphylococcal enterotoxins have high resistances to many gastrointestinal protease enzymes such as papain, trypsin, pepsin and rennin (Haffner *et al*,1996).

The most common effect of SEA was hyperactivating cells of the immune system. The bridging of TCR with MHC II molecules by superantigens triggers intracellular signaling resulting in excessive release of proinflammatory mediators and massive polyclonal T-cell proliferation. The early inducers by SEA are:- tumor necrosis factor α (TNF- α), interleukin 1 (IL-1), interleukin 2 (IL-2), interferon gamma (IFNγ) and macrophage. All of these elements promote fever, inflammation, and multiple organ injury (Krutmann *et al* ,1989). The signal transduction

pathways for staphylococcal superantigens-induced toxicity downstream from TCR/major histocompatibility complex (MHC) ligation and interaction of cell surface co-stimulatory molecules include the cytokine receptor signaling leading to activating nuclear factor kB (kappa-light-chain-enhancer of activated B cells)[24]. The nuclear factor-kB (NF-kB) is a primary

GADPH		NF-KB		Normalization			Folding		
Sample	Avg. Ct	Samples	Avg. Ct	CtΔ	CtΔΔ	$2^{-\Delta\Delta Ct}$	Control	Treated	
$Control_1$	17.2	$Control_1$	30.6	13.375	-0.185	1.1	1.0	9.07*	
$Control_2$	16.9	$Control_2$	30.6	13.625	0.065	1.0			
$Control_3$	17.1	$Control_3$	30.8	13.68	0.12	0.9			
$Treated_1$	18.2	$Treated_1$	28.8	10.65	-2.91	7.5			
$Treated_2$	17.9	$Treated_2$	28.6	10.725	-2.835	7.13			
$Treated_3$	17.8	$Treated_3$	27.7	9.91	-3.65	12.6			
Mean ± SE for control group= 0.058 Mean ± SE for treated group= 1.8							*P value* =0.045		

regulator of inflammatory response, controlling both the innate and adaptive immune systems. NF-kB proteins are present in the cytoplasm in association with inhibitory proteins known as inhibitors of NF-kB (IkBs). After activation by a wide range of stimuli, such as tumor necrosis factor α (TNFα), interleukin 1β (IL1β), pathogens, T-cell activation signals, growth factors, and stress inducers, IkB proteins become phosphorylated by I-kB-kinase β (IKBKβ) and are subsequently degraded by the proteasome, which allows NF-kB proteins to translocate to the nucleus and activate the transcription of a large number of genes, including proinflammatory cytokines, chemokines, and adhesion molecules (Krakauer, 2019).

Table (2):- showing the folding time differences in control and treated groups, (p value =0.045), Gene expression of NF-KB increasing after SEA injection in rabbits

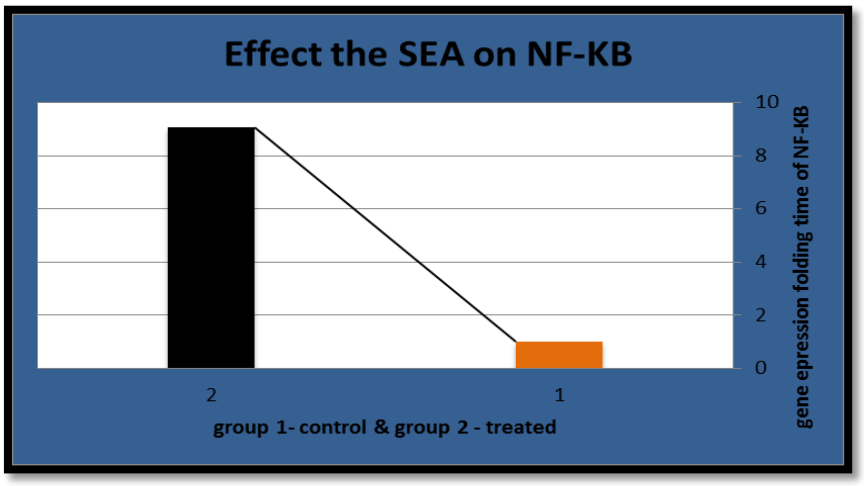

Figure (1):- showed the differences in folding time of NFKB between the control and treated group injected by SEA (p =0.045)

CONCLUSION

Staphylococcal enterotoxins A has direct effect on NF-KB gene expression. SEA was bound to MHC class II and T cell that leading to releasing more of cytokines that leading to activation NF-KB gene expression.

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