

## ROLE OF *STAPHYLOCOCCUS SAPROPHYTICUS* PEPTIDOGLYCAN IN LIVER AND KIDNEY DYSFUNCTIONS

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### Abstract

The effect of peptidoglycan extracted from *Staphylococcus saprophyticus* on liver and kidney functions was studied in rabbits, by means of injecting a group of Albino white rabbits intravenously with 6 mg / ml of partially purified *S. saprophyticus* S<sub>67</sub> peptidoglycan extract. The levels of urea, creatinine, glucose, GOT, GPT, alkaline phosphatase were estimated. The results showed a significant increase ( $P \leq 0.05$ ) in those parameters in exception of alkaline phosphatase since it developed a significant ( $P \leq 0.05$ ) decrease, however such results may indicate that the peptidoglycan caused dysfunction in both of liver and kidney.

### Introduction

*Staphylococcus saprophyticus* is a member of coagulase negative staphylococci group that cause urinary tract infections; cystitis, urethritis and pyelonephritis in young women (1), Bacteremia (2), endophthalmitis (3), wound infection, eczema (4) and respiratory tract infections (5). This bacteria has the ability to produce urease which is considered as one of the important factors in establishing urinary tract infections (6).

The rigidity of bacterial cell wall is attributed to a macromolecule known as peptidoglycan; a complex polymer composed of alternating series of two major subunits, N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). Attached to each NAM a string of tetrapeptide chain, cross linkages can form between those chains (7).

Peptidoglycan extracted from staphylococci and streptococci is known to cause septic shock in lab animals characterized by fever, inflammatory reactions, thrombocytopenia and multiple organ dysfunction syndrome (8,9). Recently, it was demonstrated that the intranasal administration of peptidoglycan from *S. aureus* to mice resulted in acute pulmonary inflammation (10). Moreover, it is characterized by having many bioactivities, it can activate leukocytes, generate proinflammatory cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), IL-6, and cause systemic inflammatory response syndrome (11). Peptidoglycan was also shown to mediate platelet aggregation in *Staphylococcus aureus*

septicemia, induce tissue factor (TF) in monocytes, and display procoagulant activity (12), and stimulation of polymorphonuclear cells, induce mast cells to produce histamine, and increase the permeability of blood vessels (13). Due to its high molecular weight, it has antigenic capacity hence elicit acute and chronic immune response (14).

The present study aimed to investigate the role of peptidoglycan extracted from *S. saprophyticus* in organs dysfunctions *in vivo*.

### Materials and Methods

#### Animals

Nine Albino white rabbits aged 2–3 months weighing 2.5- 3 kg were obtained from the animal house of Department of Biology, College of Science, University of Baghdad, divided into 3 groups as triplicates.

#### Isolation

*S. saprophyticus* was isolated as mentioned earlier and the isolate *S. saprophyticus* S<sub>67</sub> was chosen because of its multi drug resistance capacity (15).

#### Peptidoglycan extraction

The peptidoglycan of *S. saprophyticus* S<sub>67</sub> was extracted in accordance to the method described by De-Jonge (16) as follows:

One liter of culture was quickly chilled in an ice /ethanol bath until the temperature dropped below 10 C. the cells were harvested by centrifugation for 10 min at 16 xg (4° C) and subsequently transferred into 4% (final concentration ) boiling sodium dodecyl sulfate (SDS). The cells were boiled for 0.5 h and the

cell walls were then concentrated by centrifugation for 10 min at 30,000 xg. The precipitate was washed with distilled water. Cell walls were broken with glass beads (0.2 mm) on a vortex at maximal speed at 4°C for 15 min. the suspension was centrifuged at 2,000 xg, and after removal of the supernatant, the pellet was treated with glass beads as described above. The collected broken walls were centrifuged at 40, 000 xg (15), and the pellet was treated at 37°C in 100 mM Tris-HCl (pH7.5) with  $\alpha$  amylase (sigma; 100  $\mu$ g/ml). After 2 h, DNase (sigma; 10  $\mu$ g/ml) and RNase (sigma; 50  $\mu$ g/ml) were added with 20 mM (final concentration)  $MgSO_4$  and the incubation was prolonged for another 2h. Finally, the suspension was treated with trypsin (sigma; 10  $\mu$ g/ml) in the presence of 10 mM  $CaCl_2$  (final concentration) for 16 h. The enzymes were inactivated by boiling for 15 min in 1% (final concentration) SDS. The walls were collected by centrifugation as described above and washed two times with distilled water, once with 8 M LiCl, once with 100 mM EDTA, and then two times with distilled water before being washed with acetone. Finally, the broken walls were resuspended in distilled water. Five mg of broken walls were stirred in 2 ml of hydrofluoric acid (49%) for 48 h at 4°C. The peptidoglycan was recovered by centrifugation at 50, 000 xg for 45 min and washed two times with distilled water once with 100 mM Tris-HCl (pH 7.5), and finally again two times with distilled water. The peptidoglycan was collected in 0.05 % sodium azide at 4°C.

### Injection protocol

The first and second groups of rabbits were injected, intravenously, with 6 mg / ml of partially purified *S. saprophyticus* S<sub>67</sub> peptidoglycan extract, while the third group was injected with one ml of phosphate buffer saline pH 7.2 which is considered as a control.

A day later, the blood was drawn from the heart by stabbing via disposable syringe from the first and third groups. Another blood specimen was collected after seven days from the second group. The blood samples were incubated in 37°C for 15 min. thereafter; the sera were separated by centrifugation at 3500 rpm for 10 min. The levels of urea, creatinine, glucose, GOT (Glutamic

Oxaloacetic Acid), GPT (Glutamic Pyruvate Transaminase) and alkaline phosphatase (using kits which purchased from bioMérieux, France) were estimated.

### Statistical analysis

Student t-test, F test and LSD were employed in this study for statistical analysis.

### Results and Discussion

The bacteria *S. saprophyticus* S<sub>67</sub> was identified via conventional biochemical tests and the peptidoglycan was extracted by using mechanical disintegration and enzymatic digestion in according to De-Jonge (16), the results agreed with Umeda *et al.* (17).

Indolence and sluggishness were noticed on the behaviour of the injected animals after the first and seventh day of injection.

Alongside, the biochemical parameters in serum showed marked significant increase ( $P \leq 0.05$ ) in urea level after one day and seven days (non significant differences  $P \geq 0.05$ ) of the injection, which indicates a glomerular filtration defect a matter may cause renal failure since the urea increases in several pathological cases such as dehydration, acute renal failure, prostatic and intestinal obstruction (18,19) and that was confirmed by the significant increase of creatinine level in both specimens (day one and day seven specimens) in comparison to the control group level. When the urea level increased in the blood the creatinine will accumulate and its concentration will increase as well especially in pyelonephritis and urinary tract obstruction (19).

Concomitantly, the liver function tests developed a significant increase ( $P \leq 0.05$ ) in GOT and GPT when compared with the control. However, the activity of alkaline phosphatase showed significant decrease ( $P \leq 0.05$ ), which reflects dysfunction of liver due to hepatocellular damage (19).

An increase in glucose level was significantly noticed after injection the rabbits with peptidoglycan extract, which signify insufficiency in insulin production from pancreas.

Upon all previous results we can reach a presumption that; a multiple organ dysfunction may perhaps occurred because of peptidoglycan injection since the

peptidoglycan is able to cause dysfunction and damage in different functions of animal body and that may be achieved via systemic inflammatory response which is responsible for the sepsis development as the biochemical parameters have confirmed and pointed to renal and hepatic dysfunction, results have strong agreement with Dekimpe and his coworkers (20).

**Table (1)**  
**Biochemical parameters<sup>1</sup> of rabbits injected with 6 mg / ml of partially purified *S. saprophyticus* S<sub>67</sub> peptidoglycan extract.**

Group	Blood urea (mg/dl)	S.Creatinine (mg/dl)	S.GOT U/L	S.GPT U/L	S.Alkaline Phosphatase (U/L)	Blood Glucose (mmol/L)
Control Group	24 ± 2a	1.6 ± 0.2 c	10 ± 1.6e	9 ± 1 e	8 ± 0.8 e	5.5 ± 0.2 d
First Group <sup>2</sup>	48 ± 3.2 b	6 ± 0.5 d	15 ± 0.2f	18 ± 1.6f	4 ± 0.5 d	11 ± 2.1 e
Second Group <sup>3</sup>	50 ± 1.4 b	4.8 ± 0.3 d	26 ± 4.6 g	23 ± 2.7 g	5 ± 1.1 d	10 ± 1.3 e

Each different letters indicate to significant differences, while the similar letters indicate to non significant differences.

<sup>1</sup> each figure in this table represents a mean of triplicate.

<sup>2</sup> after one day of injection.

<sup>3</sup> after seven days of injection.

In this perspective, the wall components (peptidoglycan and teichoic acid) of non enterotoxigenic and non endotoxinogenic *S. aureus* have the ability to impose multiple organ failure in rat and establish sepsis and septic shock due to stimulation of inflammatory response (21, 22). Such dysfunctions represented by Arterial oxygen pressure (in lung), high bilirubinemia, increase of Alanine amino transferase level (in liver), ureamia and high concentration of creatinine in plasma (in kidney), increase the concentration of lipase in plasma (in pancreas), and creatine kinase (in skeletal muscle) are the symptoms of sepsis (20).

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#### الخلاصة

درس تأثير الببتيدوكلايكان المستخلص من بكتريا *Staphylococcus saprophyticus* في وظائف الكبد و الكلى في الارانب، عن طريق حقن مجموعة من الارانب البيض في الوريد بـ 6 ملغم /مل من مستخلص ببتيدوكلايكان بكتريا *S. saprophyticus* S<sub>67</sub> المنقى جزئياً. قيست مستويات اليوريا و الكرياتينين و الجلوكوز و GPT و GOT و الفوسفاتاز القاعدي في المصل و قد اظهرت النتائج زيادة معنوية ( $P \leq 0.05$ ) في هذه المؤشرات عدا الفوسفاتاز القاعدي الذي اظهر انخفاضاً معنوياً ( $P \leq 0.05$ ). هذه النتائج قد تشير الى ان الببتيدوكلايكان قد سبب خلل في وظائف الكبد والكلى.