



Oral and Intraperitoneal Administration of B-Glucan and Its Immunomodulatory Effect Against Staphylococcus Aureus Infection In Rats

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ABSTRACT

Beta-glucan is one of the major cell wall components of yeast, fungi, seaweed and cereals. β -glucan is one of the natural products which are useful in treating and/or preventing various diseases. In this work, we used β -glucan to study its effect against Staphylococcus aureus (*S. aureus*) infection by giving it through two routes, parenteral and oral. Parenteral injection was done through intraperitoneal injection (IP) in rats in dose 0.2 ml/rat at 7 and 14 days prior to IP challenge with *S. aureus* (1×10^8 CFU/ml). Two weeks post β -glucan administration and one-week post challenge with *S. aureus*, slight non-significant changes in RBCs count in all groups were recorded. While MCV and MCH showed significant increase in IP group before and post challenge if compared with control group. MCHC showed slight significant increase in oral and IP groups post challenge than control group. Also, there was a significant increase in white blood cells, lymphocytes, monocytes, neutrophil and eosinophiles in both oral and IP groups than control group either before or after challenge with *S. aureus*. Prebiotic β -glucan showed no harmful effect on liver and kidney functions. The present data demonstrated that administration of β -glucan per os (P.O.) followed by IP injection as a treatment regimen was significant effective in enhancement of staphylococcal infection clearance in rats. Histopathology of the lungs of infected rats revealed severe bronchiolitis and peribronchiolitis while groups treated with β -glucan showed less deterioration.

Key Words: β -Glucan, Staphylococcus Aureus, Pneumonia, Hemogram, Biochemistry, Sheep, Histopathology

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INTRODUCTION

Respiratory diseases of sheep particularly pneumonia continues to be a major problem commonly encountered in sheep flocks, affecting either groups or individuals of all ages and types [1]. The disease is multifactorial because it involves a combination of infectious causes as well as predisposing environmental and managerial factors [2]. *S. aureus* is the most common cause of lung abscesses in lambs which result in poor mortality [1];

thus, causes considerable financial losses towards the lamb producers. Economic losses include unthriftiness, treatment costs and preventive measures of non-fatal cases [2, 3].

Many strains of *S. aureus* have increased resistance to different classes of antibiotics [4]. Introduction of new antibacterial agent may be followed by the development of pathogen resistance to new agents, and then increase morbidity, mortality and treatment costs [5, 6].

Therefore, new agents either for prevention or treatment of infection must be found such as immunomodulators

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which are classified into two general classes, one class is a mammalian cell product (typically protein which selectively enhances specific cells in lymphohatobiotic system [7, 8]. The second class is substances isolated and purified from microorganisms or microbial culture fluids and these immunomodulators induce non specific stimulation of lymphobiotic system producing broad spectrum effect on many hemato cells types [9, 10]. Beta-glucan is one of these compounds that can be extracted from the cell wall of yeasts, fungi, and some bacteria and grains. B- glucan has proved enhancement effect in the protection against bacteria [11] protozoan [12] and viral diseases [13].

Glucans utilize adjuvant activities through their capability to bind to specific surface carbohydrate receptors. These receptors are expressed on the monocyte-macrophage cell lineage and other antigen-presenting immunocompetent cells, e.g. dendritic cells. The attachment of glucan molecules to these receptors results in the activation of a cascade of pathways. Subsequently, it increases the production of pro-inflammatory cytokines and chemokines inducing antigen presentation and cellular co-stimulation. This process leads to enhancing of both humoral and cellular immunity [14]

Although most of the research with this substance has been done in vitro, later research indicated the oral effectiveness of purified beta-1,3 1,6 1,4-D-glucan [15].

β -Glucans possess a diversity of beneficial effects, such as lowering plasma lipids, as well as increasing salivary IgA secretion [16].

The present study aims to determine the effect of dietary prebiotic; β -Glucan; supplementation in rats infected with *Staphylococcus aureus* as a treatment either orally or injected intraperitoneal on hematological, some biochemical parameters, hisopathological changes and bacterial count.

MATERIAL AND METHODS:

This study was carried out according to guidelines for animal experimentation and approved by the institutional animal care and the committee, National Research Center Animal care unit, Dokki, Giza, Egypt.

(1) Yeast Beta glucan (Beta 1,3 /1,6 glucan was purchased from ImmunoDyne™ Inc., Texas-USA.

(2) Bacteria

Staphylococcus aureus was isolated from 20 sheep suffered from pneumonia with mucopurulent to serous nasal discharge, coughing, loud respiratory sounds, increased rectal temperature between 40.4 to 42°C, elevated respiration rate and elevated heart rate. Sterilized deep nasal swabs were used to collect samples from infected animals after cleaning the nostrils with 70% alcohol cotton swab. The samples were subjected to bacterial isolation on agar cultural and the characteristics of the isolates were studied on MacConkey and nutrient agar. The isolates on blood agar were examined for the following size of colonies, haemolytic changes. On the other hand, MacConkey agar were examined for lactose and non-lactose fermenting mucoid colonies. Primarily

the isolates were biochemically characterized by catalase and oxidase. Further secondary biochemical tests (Indole test, Methyl-Red (MR) test, Voges-Proskauer (VP) test, Citrate Utilization test, Urease test, Nitrate Reduction test, Hydrogen Sulphide production on Triple Sugar Iron (TSI) agar, coagulase (rabbit plasma), Carbohydrate Fermentation test were performed as [17].

The strain was deep frozen and stored in a cryoprotectant vial at (-80°C). At the time of experiment, bacteria were expanded in Trypticase Soy Broth for 24 h at 37°C then the bacterial colonies were counted and the number of bacterial colony forming units (CFU) per ml of stock was calculated. An inoculum of the pathogen was diluted to the desired number of (1×10^8) CFU/ml in Phosphate Buffered Saline for in vivo studies.

(3) Animals

Forty adult male wistar rats of 145-150 gm body weight were purchased from the Animal house at National Research Centre, Dokki Giza- Egypt. Animals were housed according to the National Institute of Health guidelines and were provided food and water. Rats were quarantined for 7 days prior to being entered into experiment.

Experimental design

Rats were randomly divided into four equal groups, the first group (n=10) was kept as control negative (non treated – non infected). They were injected with sterile saline and kept for 14 days without any other treatment. The second group was kept as control positive (non treated - infected) and were injected with sterile saline. The third group was injected intraperitoneal with β -Glucan 0.2 ml/rat at the 7th and 14th days while the fourth group was administrated per os the Beta- glucan 0.2 ml/rat at the same manner. After fourteen days from the start of the experiment, the 2nd, 3rd and 4th groups were challenged by one dose intraperitoneal injection of isolated strain of *Staphylococcus aureus* 1×10^8 CFU/rat, and kept under observation for one week or its death. All rats were euthanized by the end.

Blood Samples for biochemical analysis

Two blood samples were taken from all rats at 14th day and 21st after the beginning the experiment by retro-orbital venous plexus puncture. The first blood sample collected with anti-coagulant EDTA (ethylenediaminetetraacetic acid tripotassium) for erythrogram packed cell volume (pcv), Heamoglobin concentration (Hb), Red blood count (RBCs), calculated red blood indices including mean corpuscular volume (MCH), mean corpuscular heamoglobin concentration (MCHC), and leuckogram, total leucocytic count (TLC) and its differential cell. Previous tests were done using automatic cell counter.

The second blood sample was collected without anti-coagulants and processed for serum biochemical analyses. Serum samples were stored at -20°C until further biochemical analysis. This analysis includes determination of total protein [18], Albumin [19], activity of alanin aminotransferase ALT [20], total cholesterol

[21], Urea [22] and creatinin [23]. Test kits were supplied by BiouMerieux-France.

Bacterial colonies count

Two milliliters of heparinized blood were collected from the medial eye canthus vein after 24, 48 hours and after one-week post challenge with *S. aureus*. Twenty milliliter of 50°C Tryptic Soy Agar was plated into a sterile Petri plate and 1 milliliter aliquot of blood immediately added to the plate and thoroughly mixed into the agar by swirling. Plates were incubated at 37°C for 24 h, then colonies were enumerated [6].

Histopathological studies:

All rats included in the experiment either died or euthanized through cervical dislocation by a well trained person at the end of the experiment were dissected and lung were collected then preserved in 10% formalin for histopathology. Afterward, formalized tissue was sent to the pathology laboratory for microscopic examination and paraffin sections were prepared and stained with Haematoxylin and Eosin (H&E) according to [24].

Statistical analysis:

All data were subjected to statistical analysis including the calculation of the mean and standard error (Mean \pm SE). Significance between haematological and biochemical parameters in control and treated groups were evaluated by student t-test at level $p\leq 0.05$ [25] using SPSS for windows version 15.

RESULTS

Out of the 20 collected nasal swabs, thirteen clinical samples yielded pure cultures of *Staphylococcus aureus*. The organisms appeared glistening, smooth and rise and B, haemolytic on blood agar after 24 hr at 37°C. Using Gram staining, the smears showed Gram positive cocci arranged in grapes. The organisms were confirmed as *Staphylococcus aureus* by biochemical characterization and sugar fermentation tests. All isolates showed the same trend of reaction in fermenting sugars. The organisms were positive to catalase, coagulase, urease, citrate methyl red, nitrate, Voges-Proskauer (VP) tests, while Nitrate Reduction, oxidase, Hydrogen Sulphide production and indole test are negative. The organisms were able to ferment the glucose, galactose and maltose but not the xylose. While the other seven cases showed mixed infection of other bacteria such as *Klebsiella* spp., *Escherichia coli* and *Pasteurella multocida*.

Hemogram (Table 1) showed that after 2 weeks post β -glucan administration and after 1-week post challenge with *S. aureus* slight non significant change in RBCs count in all groups was recorded. While MCV and MCH showed significant increase in IP group before and post challenge if compared to control group. MCHC showed slight significant increase in oral and IP group post challenge than control group.

Leucogram (Table 1) showed significant increase in WBCs, lymphocytes, monocytes, neutrophil and eosinophiles in both oral and IP group than control group either before or after challenge with *S. aureus*. The same result was recorded for platelets count.

Serum biochemistry (Table 2) showed results of total protein, albumin, and globulin. A/G ratio recorded significant increase in serum total protein level in oral group before challenge and in IP group after challenge. While albumin and A/G ratio showed significant decrease in group 3 and 4, 2 weeks after treatment with β -glucan and in group 4, 1-week post challenge.

Serum globulin recorded significant increase two weeks post β -glucan administration in both group 3 and 4 and significant decrease one-week post challenge with *S. aureus* in group 4.

Serum AST, ALT, Urea and creatinine; showed significant decrease in AST and ALT in group 4 after one week of challenge. Non significant change in serum urea level was recorded while serum creatinine showed slight sign decrease in group 4 one-week post challenge with *S. aureus*.

The Study evaluated the blood CFU levels in rats challenged with *S. aureus* (Table 3). At 24h, 48h and one-week post challenge, β -Glucan which administered per os was able to reduce significantly the blood CFU levels in *S. aureus* infected rats than in rats injected with β -Glucan through IP or untreated infected rats. On the other hand, at 48h and one-week post challenge β -Glucan which was administered through IP injection was able to reduce significantly the blood CFU levels in *S. aureus* infected rats than untreated infected rats. These results show that the oral treatment with β -Glucan more effective than IP route

Histopathological finding (Fig 1), Lung of the control non infected (negative group) showed normal histological structure of bronchi, bronchioles and alveoli with presence of blood filling the bronchiolar and alveolar lumen as a result of the euthanasia. While in infected (positive group), lungs showed sever bronchiolitis and peribronchiolitis that characterized microscopically by intense inflammatory reaction. The lesion is associated with perivascular edema involving the media of pulmonary blood vessel with recruitment of inflammatory cells. Mainly, neutrophils fill the vascular lumen that shows congestion, endothelial swelling and focal micro thrombi formation. On other hand, the lung of rats treated with β -Glucan IP and later challenged with *S. aureus* showed moderate inflammatory reaction. There was hyperplastic proliferation of bronchiolar epithelium, congestion of blood vessels with endothelial swelling and hyperplasia of alveoli lining epithelium. The lung of orally treated group showed marked alleviation of histopathological alterations. It was restricted to mild inflammatory reaction including the peribronchiolar area with hyperplasia of peribronchiolar lymphoid aggregation.

Table 1. Hematology profile of rats post treated with β -glucan and post challenge with S. aureus Mean \pm SE.

Parameters	Intervals (days)	Groups				Significance
		G1 (Control -ve)	G2 (Control +ve)	G3 (Oral treatment)	G4 (IP injected)	
Red blood cell count ($\times 10^6/\mu\text{l}$)	14	6.95 \pm 0.20 ^{ab}	6.96 \pm 0.21 ^{ab}	7.02 \pm 0.10 ^a	6.96 \pm 0.13 ^{ab}	**
	21	6.05 \pm 0.25 ^c	6.07 \pm 0.21 ^c	6.43 \pm 0.19 ^{bc}	5.94 \pm 0.22 ^c	
Hematocrit (%)	14	36.86 \pm 1.30 ^a	36.88 \pm 1.31 ^a	37.13 \pm 0.54 ^a	38.43 \pm 1.04 ^a	NS
	21	36.07 \pm 1.20 ^a	33.43 \pm 0.47 ^b	35.95 \pm 1.18 ^{ab}	34.93 \pm 0.94 ^{ab}	
Hemoglobin (g/dl)	14	12.70 \pm 0.51 ^{ab}	12.76 \pm 0.53 ^{ab}	12.85 \pm 0.17 ^{ab}	13.68 \pm 0.36 ^a	*
	21	12.50 \pm 0.25 ^{ab}	11.73 \pm 0.03 ^b	13.07 \pm 0.09 ^a	12.67 \pm 0.20 ^{ab}	
Mean corpuscular volume MCV (fl)	14	52.85 \pm 0.75 ^c	52.94 \pm 0.79 ^c	53.05 \pm 1.41 ^c	56.8 \pm 0.55 ^{ab}	**
	21	53.06 \pm 1.40 ^c	55.13 \pm 1.23 ^{bc}	55.9 \pm 0.17 ^{abc}	58.9 \pm 0.95 ^a	
Mean corpuscular hemoglobin MC (pg)	14	18.23 \pm 0.30 ^c	18.32 \pm 0.32 ^c	18.36 \pm 0.48 ^c	20.25 \pm 0.19 ^{ab}	**
	21	18.37 \pm 0.47 ^c	19.37 \pm 0.69 ^{bc}	20.3 \pm 0.46 ^{ab}	21.43 \pm 0.59 ^a	
Mean corpuscular hemoglobin concentration MCHC (mg/dl)	14	34.56 \pm 0.44 ^b	34.58 \pm 0.46 ^b	34.63 \pm 0.05 ^b	35.65 \pm 0.14 ^{ab}	NS
	21	34.64 \pm 0.35 ^b	35.13 \pm 0.61 ^{ab}	36.4 \pm 0.98 ^a	36.37 \pm 0.88 ^a	
Red blood cell distribution width (fl)	14	40.36 \pm 1.58 ^{abc}	41.36 \pm 1.48 ^{abc}	43.8 \pm 0.16 ^{ab}	43.23 \pm 0.44 ^c	*
	21	41.34 \pm 1.47 ^{abc}	49.13 \pm 4.23 ^a	42.7 \pm 0.87 ^{bc}	47.9 \pm 1.27 ^{bc}	
Red blood cell distribution width (%)	14	25.24 \pm 1.33 ^c	25.26 \pm 1.34 ^c	23.7 \pm 1.25 ^{bc}	23.08 \pm 0.20 ^{bc}	*
	21	23.80 \pm 0.24 ^c	28.8 \pm 2.65 ^a	23.7 \pm 0.23 ^c	24.53 \pm 0.54 ^{ac}	
Platelet count ($\times 10^3/\mu\text{l}$)	14	806.90 \pm 24.30 ^b	807.4 \pm 24.40 ^b	1158.6 \pm 79.54 ^a	839 \pm 70.03 ^b	**
	21	807.60 \pm 21.60 ^b	1347.67 \pm 56.83 ^a	1308.67 \pm 63.58 ^a	1183 \pm 103.92 ^a	
Mean platelet volume (fl)	14	5.60 \pm 0.080 ^a	5.62 \pm 0.09 ^a	5.84 \pm 0.07 ^a	5.84 \pm 0.22 ^a	NS
	21	5.90 \pm 0.21 ^a	5.40 \pm 0.21 ^a	5.33 \pm 0.23 ^a	5.75 \pm 0.09 ^a	
White blood cell count ($\times 10^3/\mu\text{l}$)	14	5.28 \pm 0.56 ^d	5.10 \pm 0.58 ^d	8.75 \pm 0.29 ^b	12.73 \pm 0.29 ^a	**
	21	5.52 \pm 0.23 ^d	5.83 \pm 0.32 ^d	7.3 \pm 0.12 ^c	7.37 \pm 0.38 ^c	
Lymphocytes ($\times 10^3/\mu\text{l}$)	14	2.68 \pm 0.26 ^b	2.58 \pm 0.27 ^b	2.43 \pm 0.09 ^b	3.76 \pm 0.11 ^a	**
	21	2.70 \pm 0.21 ^b	2.60 \pm 0.21 ^b	3.9 \pm 0.06 ^a	3.39 \pm 0.21 ^a	
Monocytes ($\times 10^3/\mu\text{l}$)	14	0.28 \pm 0.09 ^d	0.29 \pm 0.03 ^d	0.33 \pm 0.02 ^{cd}	0.4 \pm 0.03 ^c	**
	21	0.29 \pm 0.04 ^d	0.52 \pm 0.04 ^b	0.66 \pm 0.02 ^a	0.37 \pm 0.02 ^{cd}	
Neutrophils ($\times 10^3/\mu\text{l}$)	14	2.30 \pm 0.27 ^d	2.2 \pm 0.28 ^d	5.91 \pm 0.21 ^b	8.45 \pm 0.18 ^a	**
	21	2.50 \pm 0.50 ^d	2.51 \pm 0.10 ^d	2.45 \pm 0.04 ^d	3.24 \pm 0.15 ^c	
Eosinophils ($\times 10^3/\mu\text{l}$)	14	0.02 \pm 0.01 ^f	0.03 \pm 0.01 ^f	0.07 \pm 0.01 ^e	0.12 \pm 0.01 ^d	**
	21	0.03 \pm 0.04 ^f	0.20 \pm 0.01 ^c	0.28 \pm 0.02 ^b	0.34 \pm 0.01 ^a	

Means with different superscripts in the same row are significantly different at $P < 0.05$.

NS = non significant. * = $P < 0.05$. ** = $P < 0.01$.

Table 2: Serum biochemistry of rats post treated with β -glucan and post challenge with S. aureus Mean \pm SE.

Parameters	Intervals (days)	Groups				Sig.
		G1 (Control -ve)	G2 (Control +ve)	G3 (Treated orally)	G4 (Treated IP)	
Total proteins (g/dl)	14	8.72 \pm 0.04 ^b	8.41 \pm 0.20 ^b	9.15 \pm 0.30 ^a	7.79 \pm 0.08 ^b	**
	21	8.88 \pm 0.14 ^a	8.87 \pm 0.11 ^a	8.49 \pm 0.19 ^a	7.51 \pm 0.31 ^b	**
Albumin (g/dl)	14	3.86 \pm 0.04 ^a	3.88 \pm 0.05 ^a	3.68 \pm 0.07 ^b	3.63 \pm 0.02 ^b	*
	21	3.20 \pm 0.18 ^b	3.22 \pm 0.02 ^b	3.27 \pm 0.13 ^b	3.53 \pm 0.03 ^a	***
Globulin (g/dl)	14	4.80 \pm 0.90 ^b	4.83 \pm 0.10 ^b	5.42 \pm 0.13 ^a	5.4 \pm 0.06 ^a	**
	21	5.31 \pm 0.20 ^a	5.65 \pm 0.13 ^a	5.22 \pm 0.18 ^a	3.98 \pm 0.29 ^b	***
Albumin /Globulin ratio	14	0.80 \pm 0.02 ^a	0.80 \pm 0.01 ^a	0.68 \pm 0.03 ^b	0.67 \pm 0.01 ^b	**
	21	0.60 \pm 0.18 ^b	0.57 \pm 0.02 ^b	0.63 \pm 0.04 ^b	0.91 \pm 0.06 ^a	***
Aspartate amino-transferase AST (IU/l)	14	285.5 \pm 5.53	255.25 \pm 3.94	238.8 \pm 4.50	284.8 \pm 18.10	NS
	21	215.7 \pm 12.30 ^b	312.9 \pm 19.5 ^a	289.2 \pm 11.8 ^a	178.9 \pm 6.56 ^b	***
Alanine amino-transferase ALT (IU/l)	14	57.8 \pm 3.93	52.2 \pm 5.43	43.8 \pm 3.43	61.37 \pm 8.6	NS
	21	62.8 \pm 5.00 ^b	99.75 \pm 7.36 ^a	67.9 \pm 5.02 ^b	40.6 \pm 3.63 ^c	***
Urea (mg/dl)	14	34.7 \pm 1.97	35.3 \pm 8.23	33.9 \pm 1.11	32.9 \pm 1.69	NS
	21	34.05 \pm 0.91	36.6 \pm 1.25	33.7 \pm 0.47	36.7 \pm 2.44	NS
Creatinine (mg/dl)	14	1.04 \pm 0.01	0.71 \pm 0.15	1.0 \pm 0.05	0.98 \pm 0.02	NS
	21	1.03 \pm 0.01	1.00 \pm 0.01 ^b	1.20 \pm 0.08 ^a	0.91 \pm 0.00 ^b	**

Means with different superscripts in the same row are significantly different at $P < 0.05$.

NS = non significant. * = $P < 0.05$. ** = $P < 0.01$. *** = $P < 0.001$.

Table 3. Effect of β -glucan treatment on blood CFU levels in rats after 24, 48 hours and after one week post challenge with S. aureus:

Intervals	G2 Control +ve	G3 (Oral treatment)	G4 (IP injected)
After 24 hours	140 ^a \pm 10.01	72 ^b \pm 10.39	136 ^a \pm 11.86
After 48 hours	92 ^a \pm 8.5	21 ^c \pm 3.48	48 ^b \pm 4.33
After one week	58 ^a \pm 3.28	14 ^c \pm 2.60	24 ^b \pm 3.46

Data were expressed as Mean \pm Standard error. Means with different superscripts in the same row are significantly different at $P < 0.05$.

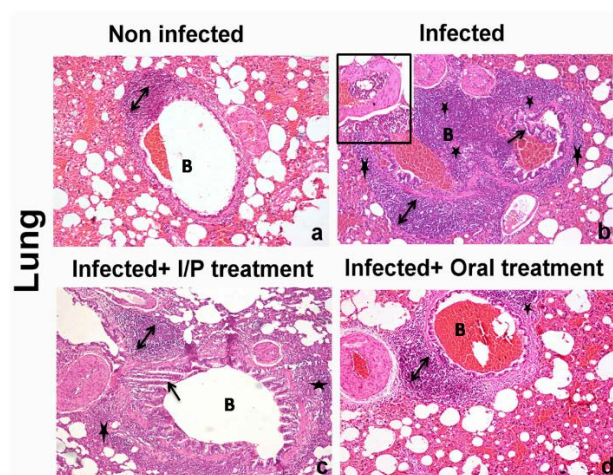


Fig. 1. H&E stained lung sections, (a) negative control group showing normal bronchiolar histological structure (B) with normal peribronchiolar lymphoid aggregation (double head arrow) (X200). (b) infected positive group showing bronchiolitis and peribronchiolitis note the intense inflammatory cells involving the bronchiolar lumen and wall (*) that merge with lymphoid aggregation (double head arrow). Focal hyperplasia of bronchial epithelium (arrow) (X200), the upper left inserted box showing endothelial swelling and inflammatory cells aggregation in vascular lumen (X400). (c) IP group showing hyperplasia of bronchiolar epithelium with less inflammatory reaction involving the peribronchial (*) with slight hyperplasia of the lymphoid aggregation (double head arrow) (X200). (d) Oral group showing focal mild inflammatory reaction involving the peribronchial tissue with hyperplasia of the associated lymphoid aggregation (X200).

DISCUSSION

Multi drug-resistant *Staphylococcus aureus* (MRSA) has been isolated from most of domestic animal species in Egypt [26, 27] led to an urgent need for new antibacterial agents. Previous studies have documented that β -Glucan are potent immunomodulator agent with effects on both innate and adaptive immunity [28].

In the present study, isolation of *Staphylococcus aureus* was recorded in 65 % (13/20) of the cases under investigation. This finding is in agreement with the previous reports [29] which demonstrated that the most common bacterial pathogens included are *Staphylococcus aureus* and *Klebsiella pneumoniae* causing pneumonia in sheep.

Staphylococcus aureus challenge in rats causing no significant changes in RBCs count, PCV, these may be due to that inflammation help in trapping of free iron and increase its storage in phagocytic cells [30, 31, 29]

Significant increase in WBCs due to *Staphylococcus aureus* was reported in acute inflammatory disease particularly bacterial infection which causes tissue injury and stimulation of cells to release growth factors, cytokines and other inflammatory mediators. So WBCs count increased and more stimulation of bone marrow to release of mature and immature neutrophils [32, 29]. In addition, the stress on animals as the result of respiratory illness leads to endogenous release of corticosteroids

which has major role in regulating the concentration of circulating WBCs [30, 29]. Eosinophilia could be result of hypersensitivity effect produced by microorganisms and release of histamine [33, 29]. In addition to generalized infection and influence of allergen which induce type 1 hypersensitivity and release of histamine could be a cause of lymphopenia [29].

Hyperproteinemia may be due to increase of globulin, while hypoproteinemia may be due to anorexia and inability of liver to synthesize protein [29]. Another suggestion that bacterial toxin causing increase of capillary permeability then plasma protein escaping in the tissue causing decrease of protein osmotic pressure in the blood and increase in tissue [34]. Albumin is also considered as a negative acute phase protein and its value declines during inflammation [35].

Decrease in albumin may activate the protective and healing function of inflammation by increase of synthesis of protective proteins such as fibrinogen, hepatoglobulin and serum amyloid acid [36].

Markers of renal function, serum concentration of urea and creatinine showed an increase one-week post challenge than that before challenge with *Staphylococcus aureus*. The increase in urea concentration may be due to acceleration catabolism of body protein as response to infection, which also causing kidney dysfunction and increase of creatinine [37, 29].

Non significant changes of ALT and AST may be attributed to protective effect of β -Glucan on liver and ending of experiment after one week of infection. The obtained result revealed that there was no effect of administration of β -glucan on AST and ALT activity and this agreement with [38, 39].

Result of protein profile showed significant increase in serum T.P and globulins and significant decrease in A/G ratio, and albumin in treated groups than control groups 14 days' post treatment before infection and these agree with [40].

Concerning to kidney function, β -Glucan has no significant effect on urea and creatinine levels and these agree with [41, 29].

The present data (Table 3) demonstrated the protective efficacy of β -glucan orally administered followed by intraperitoneal treatment regimen. It significantly enhanced the bacterial clearance of staphylococcal infection. This finding is in agreement with the previous reports [6, 42, 43, 11]. They reported the prophylactic treatment with glucan may be of potential value in combating staphylococcal infection and multiple antibiotic resistant gram positive organisms. [44] reported that β -Glucan itself can elicit broad anti-infective effects against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pneumocystis carinii*, *Listeria monocytogenes* and *Leishmania donovani*.

The histopathology of the lung isolated from infected control group revealed sever bronchiolitis and peribronchiolitis. The lesion is associated with perivascular edema involving the media of pulmonary blood vessel with recruitment of inflammatory cells. Mainly, neutrophils fill the vascular lumen that showing

congestion, endothelial swelling and focal micro thrombi formation which agree with [45] and [29]. While groups treated with β -glucan either orally or intraperitoneal showed less progression effect due to immunomodulation and immunostimulation effect of β -glucan [39,41].

Conclusion, Prebiotic β -glucan has no harmful effect on liver and kidney functions and can be considered as immunopotentiator (immunostimulant) due to its stimulation of immune system and it has ability to reduce the adverse effect of *Staphylococcus aureus* infection in rats.

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