



## The Effectiveness of $\beta$ -glucan in the Treatment of Caprine Mastitis Induced by *Candida albicans*

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### A B S T R A C T

This study evaluated the efficacy of  $\beta$ -glucan, extracted from *Candida albicans* isolated from mastitic goat milk using an alkaline-acidic method, in treating *C. albicans*-induced mastitis. Twenty lactating goats (2–4 years old, 25–30 kg) were randomly divided into five groups (Group I–Group V), with each group consisting of four goats. Sixteen goats were intramammarily inoculated with  $1.2 \times 10^7$  yeast/2 mL of virulent *C. albicans*. The groups were as follows: Group I (negative control, uninfected), Group II (positive control, infected but untreated), Group III (treated with Nystatin, 200,000 units/half udder/day intramammary for 7 days), Group IV (treated with  $\beta$ -glucan, 5 mg/mL/half udder, administered intramammary every 48 h for three doses), and Group V (treated with a combination of  $\beta$ -glucan and Nystatin;  $\beta$ -glucan was administered as in Group IV, followed 2 h later by Nystatin, 200,000 units/half udder/day for 7 days). Clinical signs, milk quality (California Mastitis Test and fungal cultures), and serum levels of IL-6 and IFN- $\gamma$  were assessed on days 0, 5, 10, 20, 30, and 40 post-inoculation. The results showed that the positive control group exhibited persistent mastitis symptoms throughout the study. Goats treated with  $\beta$ -glucan alone (Group IV) demonstrated significant symptom reduction and fungal elimination by day 15. The combination therapy group (Group V) achieved similar improvements by day 25. Serum IL-6 and IFN- $\gamma$  levels were significantly elevated in the positive control group, while the  $\beta$ -glucan-treated group showed a substantial reduction in these inflammatory markers, indicating its potential as a standalone antifungal therapy. The Nystatin group (Group III) and the combination group (Group V) also exhibited reduced cytokine levels, although these were higher than those observed in the  $\beta$ -glucan and negative control groups. This study confirms the potential of  $\beta$ -glucan as an effective treatment for *C. albicans*-induced mastitis in goats. Its ability to lower inflammatory cytokine levels and eliminate fungal infections highlights its promise as a therapeutic option, particularly in combination with Nystatin, for managing fungal mastitis.

**Keywords:** caprine mastitis, mycotic mastitis, *C. albicans*, beta-glucan, IL-6, IFN- $\gamma$

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### INTRODUCTION

Mastitis is the inflammation in the mammary glands' parenchyma. It is defined by a number of physical and chemical changes in the milk as well as pathologic abnormalities in the glandular tissue (1). Goats are among the oldest domesticated farm animals and make a very valuable contribution in developing countries, particularly

poor ones, where goat milk can help improve millions of people's nutrition (2).

There have been numerous reports of fungi as mastitis-causing agents. Even though yeasts and molds are abundantly present in nature, mycotic mastitis in ruminants is typically attributed to yeasts (3, 4). An opportunistic pathogen among yeasts is *Candida (C.) albicans* (5). Fungal mastitis in ruminants is most frequently caused by *C. albicans* (6). Beta glucan ( $\beta$ -glucan)

is polysaccharide in the wall of algae, bacteria, fungi, yeast, and plants (7, 8). It belongs to a class of  $\beta$ -glycosidic polysaccharides (9) because of their varied chemical structures, possessing a wide range of biological properties (10).

$\beta$ -glucans are not produced by cells of mammals; however, they are identified via mammalian cells as pathogen-associated molecular patterns (PAMPs) by recognition receptors and hence operate as biological reaction modifier (11).  $\beta$ -glucan exhibits many medicinal properties such as antifungal activity (12) and reducing mastitis (13). When ewes received  $\beta$ -1, 3-glucan subcutaneously, their mammary bacterial count decreased, and their milk somatic cell count (SCC) increased more quickly (14). According to Persson Waller and Colditz (15), injecting  $\beta$ -1,3-glucan into the udder of non-lactating ewes caused a noticeable movement of macrophages and lymphocytes. Additionally, injection of  $\beta$ -1,3-glucan into the cow's udder during the dry period and after drying off enhanced the numbers of certain mononuclear cell types in mammary secretions (16).

A preventative and/or therapeutic benefit against udder infections using intramammary  $\beta$ -1,3-glucan infusion has not been shown to exist in goats. Therefore, the main objective of the current experiment was assessing intramammary infusion of  $\beta$ -1,3-glucan into lactating udder that infected with *C. albicans* can stimulate the clearance of this infection.

The preventative and/or therapeutic benefits of intramammary  $\beta$ -1,3-glucan infusion against udder infections have not been demonstrated in goats. Therefore, the primary objective of the current experiment was to assess whether the intramammary infusion of  $\beta$ -1,3-glucan into the udders of lactating goats infected with *C. albicans* could help overcome this infection.

## MATERIALS AND METHODS

### Ethical Statement

Ethical approval was granted according to the local committee of care and use of the animal in research at the College of Veterinary Medicine, University of Baghdad (P.G/1302 at 19/11/2023) before starting this study.

### *C. albicans* Isolate

The *C. albicans* isolate was obtained from caprine mastitis in our earlier investigation (17), and it was analyzed both macroscopically and microscopically.

### Extraction of $\beta$ -glucan from *C. albicans*

The same *C. albicans* isolate was used for  $\beta$ -glucan extraction, following the method described by Pengkumsri et al. (7) and Bacha et al. (18), utilizing an alkaline-acidic extraction technique. The dry weight of  $\beta$ -glucan produced by this method was 7.47 g per 150 g (4.98%). The  $\beta$ -glucan was then suspended at 5 mg/mL for use in the experiment, as outlined by Inchainri et al. (16) and Persson-Waller et al. (19).

## Determination of *C. albicans* Inoculum

*C. albicans* was cultured on Sabouraud dextrose agar (SDA) (HiMedia, China) supplemented with 0.03% chloramphenicol. After incubation for 5–6 days at 37°C, the growth was harvested using sterile phosphate-buffered saline (PBS, pH 7.4) containing 0.05% Tween-20. The resulting suspension was homogenized with a magnetic stirrer, and the blastoconidia concentration was adjusted to  $1.2 \times 10^7$  cells/2 mL using a hemocytometer (20, 21).

## Animals and Experimental Design

Twenty healthy local-breed goats (*Capra hircus*), aged 2–4 years and weighing 25–30 kg, were used in the study. All animals were free of mastitic pathogens and clinically free from other infectious diseases. Additionally, twenty-seven kids were separated from their dams 21 days prior to the start of the experiment. The study was conducted at the College of Veterinary Medicine, University of Baghdad. During the 21-day acclimatization period, the goats were housed in individual cages, provided with concentrated feed and hay, and had *ad libitum* access to water.

The animals were randomly divided into five groups (Group I-Group V), with each group consisting of four goats. The procedure for infection followed the method described by (20). The right half of the udder in Groups II, III, IV, and V was intramammarily injected with 2 mL containing  $1.2 \times 10^7$  yeast of *C. albicans* inoculum, while the left half of the udder served as the control. Treatment began on day 3 post-*C. albicans* inoculation as follows: Group I (Negative Control), uninfected, receiving 2 mL of sterile PBS in the right mammary gland; Group II (Positive Control), infected with *C. albicans* and untreated; Group III, treated with 5 mL of 200,000 units of Nystatin daily for 7 days; Group IV, treated with 5 mL of 5 mg/mL  $\beta$ -glucan every 48 h for three doses; Group V, treated with 5 mL of 5 mg/mL  $\beta$ -glucan every 48 h, followed 2 h later by 5 mL of 200,000 units of Nystatin daily for 7 days. All animals were closely observed daily for changes in udder health, including mammary gland palpation and milk secretion appearance. Milk samples were collected before and after inoculation and tested for mastitis using the California Mastitis Test (CMT). Additionally, milk samples from the infected and control halves were cultured on SDA at 0, 3, 5, 8, 10, 15, 20, 25, 30, 35, and 40 days following the start of the experiment (22).

## Clinical Observations

Regular clinical examinations were conducted to evaluate the overall health of the animals, including rectal temperature, heart rate, respiration rate, and appetite. Udder health was assessed through palpation of the mammary gland, observation of udder secretion appearance, and CMT results. Visible changes in the mammary gland and milk were recorded as clinical signs.

## IL-6 and IFN- $\gamma$ Measurement

Blood samples were collected from the jugular vein on days 0, 5, 10, 20, 30, and 40 of the study. The samples were allowed to coagulate at room temperature for 30 min before being centrifuged at 5000 rpm for 10 min. The

resulting serum was collected and stored in a freezer until further analysis (23). The concentrations of IL-6 and IFN- $\gamma$  were measured using a goat IL-6 ELISA kit (Cloud-Clone Corp, USA) and a goat IFN- $\gamma$  ELISA kit (SunLong Biotech Co., Ltd., China). Optical density was measured at 450 nm using a HumanReader HS ELISA reader (Human, Germany), and the levels of IL-6 and IFN- $\gamma$  were calculated based on the standard curves provided with each kit (24).

### Statistical Analysis

The Statistical Analysis System (SAS) software (25) was used to analyze the effects of treatment type and time on the study parameters. Two-way Analysis of Variance (ANOVA), followed by the least significant difference (LSD) test, was employed to compare the means.

## RESULTS AND DISCUSSION

### Physical Examinations

Physical examinations of animals were performed 72, 48, and 24 h before *C. albicans* infection for five groups, and 24, 48, and 72 h after *C. albicans* infection for 4 groups and compared to groups I. All goats exhibited a slight but consistent rise in temperature, heart rate, and respiration rate (data not shown) compared to the negative control group (Group I). However, the results indicated that the infected goats did not display any significant systemic response during the experiment.

### Clinical Changes of Udder

The clinical state of the udder was assessed based on the shape, size, and symmetry of the mammary glands and supramammary lymph nodes. Swelling and enlargement of the lymph nodes, as well as changes in milk appearance, were observed in the infected goats. In Group I (Negative Control), udder production remained normal, with negative CMT scores and no observable changes in the udder throughout the study period. In Group II (Positive Control), milk color changed to yellow, milk output decreased, and

CMT scores reached 3. Enlargement of the right supramammary lymph nodes was observed, likely due to *C. albicans* invasion of the mammary tissues. The udder showed a decrease in size, and *C. albicans* was isolated from the mammary gland on days 0, 3, 5, and 8 (Table 1, Figure 1 A). In Group III (Nystatin Treatment), CMT scores reached 3 by day 3 post-infection. Milk color changed, and enlargement of the right supramammary lymph nodes was noted on day 5, with a gradual return to normal by day 35 post-treatment. Milk samples from this group showed no *C. albicans* growth after treatment (Table 1). In Group IV ( $\beta$ -glucan Treatment), mastitis was observed by day 3, followed by a progressive return to normal, with negative CMT and mycology culture results by day 15. Normal milk was restored three days after the final treatment, with no fungal isolation from milk samples on days 15, 20, 25, 30, 35, and 40 (Table 1, Figure 1 B). In Group V (Combined Treatment), mastitis was observed by day 3, with recovery evident by day 25. Milk returned to normal on day 25, and no yeast was detected from milk samples after the final treatment (Table 2).

**Table 1.** Mycological cultures of lactating goat milk samples following the last treatment

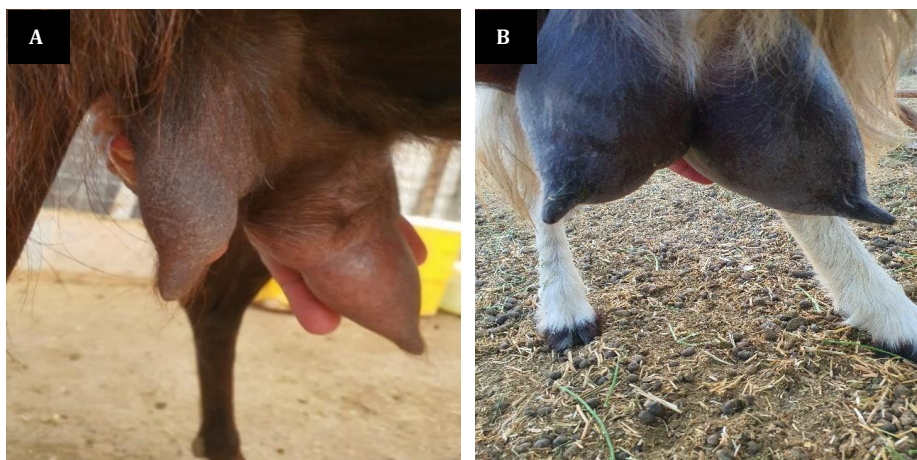
Days	Group I	Group II	Group III	Group IV	Group V
0 day	-ve	-ve	-ve	-ve	-ve
3 days	-ve	+ve	+ve	+ve	+ve
5 days	-ve	+ve	+ve	+ve	+ve
8 days	-ve	+ve	+ve	+ve	+ve
10 days	-ve	+ve	+ve	+ve	+ve
15 days	-ve	+ve	+ve	-ve	+ve
20 days	-ve	+ve	+ve	-ve	+ve
25 days	-ve	+ve	+ve	-ve	-ve
30 days	-ve	+ve	+ve	-ve	-ve
35 days	-ve	+ve	+ve	-ve	-ve
40 days	-ve	+ve	-ve	-ve	-ve

Group I: Negative control (uninfected, received sterile PBS). Group II: Positive control (infected with *C. albicans*, untreated). Group III: Treated with Nystatin (200,000 units/half udder/day intramammary for 7 days). Group IV: Treated with  $\beta$ -glucan (5 mg/mL/half udder intramammary every 48 h for three doses). Group V: Treated with  $\beta$ -glucan and Nystatin ( $\beta$ -glucan administered as in Group IV, followed 2 h later by Nystatin as in Group III)

**Table 2.** Clinical Observations and CMT Test Results Across Experimental Groups

Day	Group I	Group II	Group III	Group IV	Group V
0	No systemic signs	No systemic signs	No systemic signs	No systemic signs	No systemic signs
5	No visible disease signs	CMT 3 in 3 cases, milk color change in 1 case, enlargement of LLN in all cases, drop in milk output, fibrosis begins, udder size decreases	CMT 3 in 2 cases, milk color change in 2 cases	CMT 3 in 2 cases, milk color change in 2 cases	CMT 3 in 1 case, milk color change in 3 cases
10	No visible disease signs	CMT 3 in 3 cases, milk color change in 1 case, LLN enlargement in all, drop in milk output, fibrosis, decreased udder size	CMT 3 in 2 cases, milk color change in 2 cases, LLN enlargement in all, drop in milk output	CMT 3 in 2 cases, trace in 2 cases	CMT 3 in 1 case, milk color change in 3 cases, LLN enlargement
20	No visible disease signs	Fibrosis, decreased udder size	All cases converted to CMT score 2	No visible mastitis signs	All cases converted to CMT score 2
30	No visible disease signs	Fibrosis	All cases converted to CMT score 2	No visible mastitis signs	No visible mastitis signs
40	No visible disease signs	Fibrosis	No visible mastitis signs in 2 cases, CMT score 1 in 1 case	No visible mastitis signs	No visible mastitis signs

CMT: California Mastitis Test, LLN: lateral lymph node



**Figure 1. (A)** A photograph from goat in Group II (Positive Control) showing the morphological changes in the infected right udder half (red arrow) of a goat, with a significant reduction in size compared to the non-infected left udder half (white arrow) after 7 days post-inoculation with *Candida albicans*. The reduction in size and asymmetry reflects the progression of mastitis and associated fibrosis in the infected gland. **(B)** A photograph of an udder from a goat in Group IV on day 20 post-inoculation with *C. albicans*. The animal was treated with 5 mL of 5 mg/mL  $\beta$ -glucan administered intramammarily every 48 hours for three doses. The udder appears restored to normal size and symmetry, demonstrating the therapeutic efficacy of  $\beta$ -glucan in resolving mastitis and reversing associated tissue damage

The cure rates for Groups III, IV, and V, infected with *C. albicans*, were 100%, albeit achieved at different time points. After intramammary infusion with  $\beta$ -glucan, *C. albicans* colonies were completely absent from the milk cultures. Fibrosis, likely caused by a chemotactic response to factors released by damaged cells, led to swelling of surrounding connective tissue. This tissue progressively increased in quantity and deposited in the per-glandular and periductal regions, ultimately obliterating the lumen, acini, and cistern, impairing mammary gland vascularization. The advancement of fibrous tissue contributed to gland atrophy and dysfunction (26).

The findings of the present study align with those of Singh et al. (20), who reported a sharp and progressive decrease in milk supply from infected udder halves starting on day 1 post-infection. Within 24 hours, infected right udder halves developed clinical mastitis in two goats, characterized by yellow milk, enlarged udder halves compared to contralateral controls, and positive CMT results (score 3+). Similarly, the present investigation corroborates findings by Al-Dujaily and Mahmood (27), who observed a strong positive CMT score (3+) in all examined goats.

Jand and Dhillon (28) isolated *C. albicans* from mastitic udders within six hours post-inoculation. Several studies, including (20, 29, 30), have also reported the onset of mastitis within 24 hours of yeast inoculation. The present study supports the capability of *Candida* to induce mastitis when applied at sufficient doses, consistent with (31, 20).

No systemic disturbances were observed during this investigation, consistent with findings by (31). However, other researchers (30) reported systemic disturbances, such as fever and anorexia, in cases of candidal mastitis. Clinical mastitis was associated with milk discoloration in all affected goats in this study, contrary to Singh et al. (20), who found no change in milk color or quality in the

uninfected udder halves of experimental and control animals.

Milk samples revealed alterations in appearance within 24 h post-inoculation. Mastitis was fully induced by day 3, as confirmed by positive CMT results (score 3), aligning with the findings of (32, 33).

One plausible explanation for the persistence of mastitis in some cases is the formation of biofilms, which facilitate fungal survival by integrating into mammary epithelial cells. Additionally, the absence of *C. albicans* in milk following  $\beta$ -glucan treatment suggests its ability to eradicate the fungus, potentially through direct action on fungal cells. These findings align with Alanni (34), who reported recovery in infected mammary glands treated with  $\beta$ -glucan extracted from *C. albicans*. Moreover, this agrees with studies by (35), demonstrating the antimicrobial properties of  $\beta$ -glucan against fungi and yeast.

Alanni (34) further noted that  $\beta$ -glucan extracted from *C. albicans* was more effective in treating mastitis in mice compared to  $\beta$ -glucan extracted from *S. cerevisiae*. Similarly, Buddle et al. (14) reported that subcutaneous  $\beta$ -glucan injections in sheep reduced bacterial load in the udder and enhanced macrophage migration. Other studies (36, 13, 37) demonstrated that  $\beta$ -1,3-glucan increases the recruitment of leukocytes and macrophages to the mammary gland and has protective effects against shipping fever in calves.

$\beta$ -1,3-glucan may induce specific inflammatory and immunological responses in the mammary gland during dry-off or constant dry phases. Repeat injections could prolong its effects, as suggested by (16). Jameel and Yassein (38) found that  $\beta$ -glucan extracted from *C. albicans* elicited a strong recovery response in mice infected with candidiasis at a concentration of 50 mg/kg administered intraperitoneally.

The combination of  $\beta$ -glucan with Nystatin resulted in complete recovery and the absence of *C. albicans* in the milk by day 25. This suggests a synergistic or complementary effect, as the two agents may enhance each other's antifungal activity, potentially reducing the required doses and minimizing adverse effects.

### IL-6 and IFN- $\gamma$ Levels

The mean IL-6 levels in the serum are presented in Table 3. Group IV exhibited significantly lower IL-6 levels ( $P < 0.05$ ) compared to Group II. Groups III and V also showed significantly reduced IL-6 levels compared to Group II, though they remained higher than those in Groups I and IV. By day 10, the IL-6 concentration in Group IV was

significantly reduced ( $1110.69 \pm 53.84$ ) compared to the infected group ( $1858.04 \pm 54.07$ ), with a continuous decrease until day 40. Significant differences were observed between Groups III and V from day 10 to day 30 ( $P < 0.05$ ).

The mean IFN- $\gamma$  levels are presented in Table 4. Group IV showed significantly lower IFN- $\gamma$  levels ( $P < 0.05$ ) compared to Group II. Similarly, Groups III and V exhibited reduced IFN- $\gamma$  levels compared to Group II, though they remained higher than those in Groups I and IV. By day 10, Group IV displayed a significant reduction in IFN- $\gamma$  ( $78.16 \pm 1.22$ ) compared to the infected group ( $178.60 \pm 1.39$ ), with sustained decreases through day 40.

**Table 3.** Differences in IL-6 concentrations (pg/mL) in five groups measured by ELISA method over different periods

Days	Group I	Group II	Group III	Group IV	Group V
0	248.03 $\pm$ 9.39 <sup>Aa</sup>	273.95 $\pm$ 15.08 <sup>Ba</sup>	258.89 $\pm$ 13.87 <sup>Ea</sup>	248.06 $\pm$ 4.91 <sup>Da</sup>	274.48 $\pm$ 12.81 <sup>Ea</sup>
5	241.92 $\pm$ 16.47 <sup>Ac</sup>	1963.07 $\pm$ 35.33 <sup>Aa</sup>	1659.8 $\pm$ 145.0 <sup>Ab</sup>	1711.89 $\pm$ 63.04 <sup>Ab</sup>	1783.54 $\pm$ 32.0 <sup>Ab</sup>
10	174.09 $\pm$ 26.70 <sup>Ae</sup>	1858.04 $\pm$ 54.07 <sup>Aa</sup>	1546.52 $\pm$ 119.23 <sup>Ab</sup>	1110.69 $\pm$ 53.84 <sup>Bd</sup>	1351.97 $\pm$ 32.79 <sup>Bc</sup>
20	264.59 $\pm$ 14.46 <sup>Ae</sup>	1892.82 $\pm$ 62.83 <sup>Aa</sup>	1309.66 $\pm$ 19.03 <sup>Bb</sup>	660.21 $\pm$ 31.07 <sup>Cd</sup>	869.99 $\pm$ 27.30 <sup>Cc</sup>
30	220.23 $\pm$ 21.64 <sup>Ae</sup>	1857.62 $\pm$ 60.96 <sup>Aa</sup>	1054.92 $\pm$ 38.98 <sup>Cb</sup>	336.92 $\pm$ 8.26 <sup>Dd</sup>	775.34 $\pm$ 32.62 <sup>Cc</sup>
40	232.27 $\pm$ 20.53 <sup>Ac</sup>	1769.95 $\pm$ 160.11 <sup>Aa</sup>	629.72 $\pm$ 53.97 <sup>Db</sup>	191.65 $\pm$ 11.21 <sup>Dc</sup>	546.35 $\pm$ 19.11 <sup>Db</sup>

Means ( $\pm$ SEM) having different small letters within the same row and different capital letters within the same column differ significantly ( $P \leq 0.05$ ). LSD = 152.38

**Table 4.** Variations in IFN- $\gamma$  Levels (pg/mL) evaluated by ELISA in five groups over different periods

Days	Group I	Group II	Group III	Group IV	Group V
0	38.54 $\pm$ 1.36 <sup>Aa</sup>	39.16 $\pm$ 8.91 <sup>Ca</sup>	36.54 $\pm$ 9.14 <sup>Ca</sup>	36.27 $\pm$ 6.36 <sup>Ca</sup>	21.38 $\pm$ 0.39 <sup>Ea</sup>
5	29.83 $\pm$ 4.98 <sup>Ac</sup>	171.52 $\pm$ 2.29 <sup>Aa</sup>	136.29 $\pm$ 13.61 <sup>Aab</sup>	128.86 $\pm$ 17.88 <sup>Ab</sup>	116.57 $\pm$ 16.22 <sup>Ab</sup>
10	12.97 $\pm$ 1.57 <sup>Ae</sup>	178.60 $\pm$ 1.39 <sup>Aa</sup>	95.59 $\pm$ 1.84 <sup>Bb</sup>	78.16 $\pm$ 1.22 <sup>Bd</sup>	89.39 $\pm$ 0.51 <sup>Bc</sup>
20	32.92 $\pm$ 7.33 <sup>Ad</sup>	166.07 $\pm$ 12.88 <sup>ABa</sup>	86.29 $\pm$ 3.50 <sup>Bb</sup>	53.44 $\pm$ 1.82 <sup>BCcd</sup>	67.64 $\pm$ 3.36 <sup>CDbc</sup>
30	17.62 $\pm$ 4.02 <sup>Ac</sup>	135.11 $\pm$ 5.69 <sup>Ba</sup>	67.11 $\pm$ 1.45 <sup>BCb</sup>	21.52 $\pm$ 3.28 <sup>Cc</sup>	63.22 $\pm$ 1.69 <sup>CDb</sup>
40	23.54 $\pm$ 6.09 <sup>Ac</sup>	134.14 $\pm$ 26.48 <sup>Ba</sup>	62.75 $\pm$ 2.07 <sup>BCb</sup>	21.40 $\pm$ 0.47 <sup>Cc</sup>	52.71 $\pm$ 6.22 <sup>DEbc</sup>

Means ( $\pm$ SEM) having different small letters within the same row and different capital letters within the same column differ significantly ( $P \leq 0.05$ ). LSD = 36.924

The movement of neutrophils from the bloodstream to mammary gland tissue occurs as a response to pro-inflammatory cytokines (39, 40). IL-6 is a pleiotropic cytokine with diverse functions, including host protection through immunological responses, regulation of hematopoiesis, and management of inflammation (41, 42).

IFN- $\gamma$  plays a critical role in directing leukocytes to sites of infection by regulating the production of chemokines and specific adhesion molecules. Additionally, IFN- $\gamma$  antimicrobial effectors enhance receptor-mediated phagocytosis and promote microbial destruction in neutrophils and macrophages (43).

This study suggests that  $\beta$ -glucan activates immune cells such as neutrophils, macrophages, and dendritic cells, leading to the production of immune-enhancing cytokines (IL-6 and IFN- $\gamma$ ). These cytokines are pivotal in recruiting and activating the immune system.  $\beta$ -glucan enhances the host immune response by stimulating the complement system, macrophage activity, and natural killer (NK) cell function. Cellular responses elicited by mushroom-derived and other  $\beta$ -glucans depend on their specific interactions with multiple cell surface receptors, including complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (bGR). As an immunostimulant,  $\beta$ -glucan works by activating macrophages and NK cell cytotoxicity (44).

$\beta$ -glucan has been shown to stimulate macrophages to produce immune-stimulating cytokines, including IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$ , thereby exerting a favorable immunomodulatory effect. This is characterized by a marked elevation of TNF- $\alpha$ , IL-6, IFN- $\gamma$ , and IL-2 levels (45, 35). Findings from (46) revealed significantly higher IL-6 concentrations in the control group compared to the  $\beta$ -glucan-treated groups. Prophylactic injection of  $\beta$ -glucan also improved septic conditions by reducing pro-inflammatory cytokine levels and increasing anti-inflammatory cytokine levels.

Sonck (47) demonstrated that  $\beta$ -glucan stimulation of monocyte-derived dendritic cells (curdlan) resulted in elevated levels of pro-inflammatory cytokines IL-6, IL-10, and IL-12/IL-23. Similarly, (16) reported that  $\beta$ -1,3-glucan triggered specific inflammatory and immunological responses in cow udders following intramammary injections during the constant dry phase or drying-off period. These findings suggest that intramammary injections of  $\beta$ -1,3-glucan could enhance the udder's defense systems against infections, particularly during initial involution.

Mahdi et al. (48) observed high levels of IFN- $\gamma$  in goats treated intramammarily with cytosine-phosphate-guanosine oligodeoxynucleotides compared to other groups. The results of this study are consistent with (49), who reported significantly higher IFN- $\gamma$  levels in infected



animals with mastitis compared to controls ( $340.21 \pm 41.61$  pg/mL vs.  $8.45 \pm 0.83$  pg/mL, respectively). Moreover, IFN- $\gamma$  levels were markedly higher in mastitis-infected mice compared to other treated groups. These findings suggest that  $\beta$ -glucan acts as an antifungal agent by eradicating *C. albicans* in caprine mammary glands within 15 days of treatment.

$\beta$ -glucan has the potential to modify the progression of *Candida albicans*-induced mastitis in goats, demonstrating therapeutic efficacy in both subclinical and clinical cases. When used in combination with the antifungal agent Nystatin,  $\beta$ -glucan exhibited enhanced effects, although its standalone use also proved effective. Additionally,  $\beta$ -glucan possesses significant immunomodulatory properties, capable of potentiating cellular immunity by activating immune cells and promoting cytokine production. These findings suggest that  $\beta$ -glucan is a promising therapeutic agent for managing fungal mastitis in goats while supporting immune system activation to enhance overall host defense.

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N/A

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- Ezzat Alnakip M, Quintela-Baluja M, Böhme K, Fernández-No I, Caamaño-Antelo S, Calo-Mata P, et al. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. *J Vet Med.* 2014;2014:659801. <https://doi.org/10.1155/2014/659801>
- Navamniraj NK, Sivasabari K, Ankitha IJ, Deepika K, Anjali MR, Akhil PR, et al. Beneficial impacts of goat milk on the nutritional status and general well-being of human beings: anecdotal evidence. *J Expe Biol Agric Sci.* 2023;11(1):1-15. [http://dx.doi.org/10.18006/2023.11\(1\).1.15](http://dx.doi.org/10.18006/2023.11(1).1.15)
- Yanuartono Y, Nururrozi, A. Indarjulianto S, Raharjo S, Purnamaningsih H, Haribowo DN. Mycotic mastitis in ruminants. *Indonesian J Anim Sci.* 2019;29(2):109-130. <https://doi.org/10.21776/ub.jiip.2019.029.02.03>
- Jameel FAR, Yassein SN. Virulence potential of *Penicillium chrysogenum* isolated from subclinical bovine mastitis. *Iraqi J Sci.* 2021;62(7):2131-2142. <https://doi.org/10.24996/ijs.2021.62.7.2>
- Hizlisoy H, Onmaz NE, Serhat Al, Karadal F, Yildirim Y, Gonulalan Z, et al. Clonal diversity and antifungal susceptibility of *Candida* spp. recovered from cow milk. *Mljekarstvo.* 2020;70(1):40-49. <https://doi.org/10.15567/mljekarstvo.2020.0104>
- Machado GP. Mastitis in small ruminants. *Anim Husb Dairy Vet Sci.* 2018;2(4):1-9. <https://doi.org/10.15761/AHDVS.1000144>
- Pengkumsri N, Sivamaruthi BS, Sirlun S, Peerajan S, Kesika P, Chaiyasut K, et al. Extraction of  $\beta$ -glucan from *Saccharomyces cerevisiae*: Comparison of different extraction methods and in vivo assessment of immunomodulatory effect in mice. *Food Sci. Technol. Campinas.* 2017;37(1):124-130. <http://dx.doi.org/10.1590/1678-457X.10716>
- Walachowski S, Breyne K, Secher T, Cougoule C, Guzylack-Piriou L, Meyer E, et al. Oral supplementation with yeast  $\beta$ -glucans improves the resolution of *Escherichia coli*-associated inflammatory responses independently of monocyte/macrophage immune training. *Front Immunol.* 2022;13:1086413. <http://dx.doi.org/10.3389/fimmu.2022.1086413>
- Vetvicka V, Teplyakova TV, Shintyapina AB, Korolenko TA. Effects of medicinal fungi-derived  $\beta$ -glucan on tumor progression. *J Fungi (Basel).* 2021;7(4):250. doi: <http://dx.doi.org/10.3390/jof7040250>
- Vlassopoulou M, Yannakoulia M, Pletsas V, Zervakis GI, Kyriacou A. Effects of fungal beta-glucans on health – a systematic review of randomized controlled trials. *Food Funct.* 2021;12(8):3366-3380. <https://doi.org/10.1039/D1FO00122A>
- Hofer M, Pospisil M. Modulation of animal and human hematopoiesis by  $\beta$ -glucans: A review. *Molecules.* 2011;16(9):7969-7979. <https://doi.org/10.3390/molecules16097969>
- Mirończuk-Chodakowska I, Kujawowicz K, Witkowska AM. Beta-Glucans from fungi: Biological and health-promoting potential in the COVID-19 pandemic era. *Nutrients.* 2021;13(11):3960. <https://doi.org/10.3390/nu13113960>
- Camilli G, Tabouret G, Quintin J. The complexity of fungal  $\beta$ -Glucan in health and disease: Effects on the mononuclear phagocyte system. *Front. Immunol.* 2018;9:673. <https://doi.org/10.3389/fimmu.2018.00673>
- Buddle BM, Pulford HD, Ralston M. Protective Effect of glucan against experimentally induced staphylococcal mastitis in ewes. *Vet Microbiol.* 1988;16(1):67-76. [https://doi.org/10.1016/0378-1135\(88\)90127-7](https://doi.org/10.1016/0378-1135(88)90127-7)
- Waller KP, Colditz IG. Effect of intramammary infusion of  $\beta$ -1,3-glucan or interleukin-2 on leukocyte subpopulations in mammary glands of sheep. *Am J Vet Res.* 1999;60(6):703-707. <https://doi.org/10.2460/ajvr.1999.60.06.703>
- Inchaisri C, Waller KP, Johannisson A. Studies on the modulation of leucocyte subpopulations and immunoglobulins following intramammary infusion of beta 1,3-glucan into the bovine udder during the dry period. *J Vet Med B Infect Dis Vet Public Health.* 2000;47(5):373-86. <https://doi.org/10.1046/j.1439-0450.2000.00357.x>
- Mahmoud SH, Yassein SN. Prevalence of mycotic mastitis and evaluation of some virulence potential of *Candida albicans* isolated from mastitic goats. *Adv. Anim. Vet. Sci.* 2023;11(9):1417-1427. <https://doi.org/10.17582/journal.aavs/2023/11.9.1417.1427>
- Bacha U, Nasir M, Iqbal S, Anjum, AA. Nutraceutical, anti-inflammatory and immune modulatory effects of  $\beta$ -glucan isolated from yeast. *Biomed Res Int.* 2017;2017:8972678. <https://doi.org/10.1155/2017/8972678>
- Persson Waller K, Grönlund U, Johannisson A. Intramammary infusion of  $\beta$ 1,3-glucan for prevention and treatment of *Staphylococcus aureus* mastitis. *J Vet Med B Infect Dis Vet Public Health.* 2003;50(3):121-127. <https://doi.org/10.1046/j.1439-0450.2003.00630.x>
- Singh P, Sood N, Gupta PP, Jand SK, Banga HS. Experimental candidal mastitis in goats: Clinical, haematological, biochemical and sequential pathological studies. *Mycopathologia.* 1997;140(2):89-97. <https://doi.org/10.1023/A:1006869422876>
- Guhad A, Jensen HE, Hau J. Experimental murine mycotic mastitis: a sensitive and lenient model for studies of antifungal chemotherapy. *FEMS Immunol Med Microbiol.* 1999;26(2):125-130. <https://doi.org/10.1111/j.1574-695X.1999.tb01379.x>
- Washinton WJ, Stephan A, William J, Elmer K, Gail W. *Konemans color Atlas and Textbook of diagnostic Microbiology*; 6th ed. Jones & Bartlett Learning Philadelphia, 2006. p.1535
- Mohammed, R.I. and Al-Samarrae IAA. Investigating the effect of three antigens of *Citrobacter freundii* on rabbit's immune response. *Iraqi J Vet Med.* 2021;45(1):56-62. <https://doi.org/10.30539/ijvm.v45i1.1043>
- Hussein DK, Al-Jowari SA, Rahmah AM. Determination of the Level of IL-6 and Vaspin in Hyperthyroid Patients Treated with Carbimazole. *Iraqi J Sci.* 2022;63(5):1909-1917. <https://doi.org/10.24996/ijs.2022.63.5.5>
- SAS. *Statistical Analysis System, User's Guide.* Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA. 2018.
- Mohanty BK, Rath PK, Panda S K, Mishra BP. Pathomorphological studies of caprine mastitic udder. *J Entomolo Zool Stud.* 2019;7(5):1208-1212. <http://www.entomoljournal.com>

27. Al-Dujaily AH, Mahmood AK. The effectiveness of biogenic silver nanoparticles in the treatment of caprine mastitis induced by *Staphylococcus aureus*. Iraqi J Vet Sci. 2021;35(III):73-78. <https://doi.org/10.33899/ijvs.2021.131415.1946>
28. Jand SK, Dhillon SS. Mycotic mastitis produced experimentally in goats. Mycosis. 1975;18(8):363-366. <https://doi.org/10.1111/j.1439-0507.1975.tb03616.x>
29. Spika M. *Candida tropicalis* as the cause of bovine mastitis. Vet Glasn. 1957;11:747-751.
30. Monga DP, Kalra DS. Observations on experimental mycotic mastitis in goats. Mykosen. 1972;15:199-205. <https://doi.org/10.1111/j.1439-0507.1972.tb02484.x>
31. Verma PC, Kalra DS. Experimental studies on mastitis in goats caused by *Candida albicans* extracts. Indian J Vet Pathol. 1983;7:21-25.
32. Ma JL, Wang JF, Wang K, Wu CX, Lai T, Zhu YH. Changes in micromineral, magnesium, cytokine, and cortisol concentrations in blood of dairy goats following intramammary inoculation with *Staphylococcus aureus*. J Dairy Sci. 2007;90(10):4679-4683. <https://doi.org/10.3168/jds.2006-868>
33. Singh V, Ram M, Roy BK, Singh KK. Effect of induced mastitis on disposition kinetics of gatifloxacin following intravenous administration in goats. J Bioanal Biomed. 2010;2(2):44-47. <https://doi.org/10.4172/1948-593x.1000020>
34. Alanni FAJ. Study The Effect of Beta-glucan Extracted from *Candida albicans* and Saccharomyces cerevisiae on The Immune Response against Penicillium chrysogenum Isolated from Bovine Mastitis in Mice [dissertation]. Baghdad, Iraq: University of Baghdad; 2021.
35. Mahmoud Amer EM, Saber SH, Abo Markeb A, Elkhawaga AA, Mekhemer IMA, Zohri ANA, et al., Enhancement of  $\beta$ -Glucan Biological Activity Using a Modified Acid-Base Extraction Method from *Saccharomyces cerevisiae*. Molecules. 2021;26:2113. <https://doi.org/10.3390/molecules26082113>
36. Pedroso M. Application of beta-1,3-glucan to prevent shipping fever in imported heifers. Arch Med Res. 1994;25(2):181
37. Małaczewska JO, Wójcik R, Jung L, Siwicki AK. Effect of Biolex  $\beta$ -HP on selected parameters of specific and non-specific humoral and cellular immunity in rats. Bull Vet Inst Pulawy. 2010;54:75-80.
38. Jameel, F.A. and Yassein, Sh.N. Effect  $\beta$  glucan extracted from *Candida albicans* on pathological changes produced by *Penicillium chrysogenum* infection in mice Iraqi Journal of Veterinary Sciences., 2023;37(4):907-914. <https://doi.org/10.33899/ijvs.2023.137775.2728>
39. Saud HM, Alaubydi MA. Effect of clinical *Klebsiella pneumoniae* extracted melanin on some immune aspects in mice. Iraqi J Agric Sci. 2019;50(1):241-247. <https://doi.org/10.36103/ijas.v50i1.301>
40. Al-Attaby, AK., Al-Lami, MQD. Role of Calcium-Regulating Hormones, Adipocytokines and Renal Function Test in The Progress of Type 2 Diabetes Mellitus in A Sample of Iraqi Patients. Iraqi Journal of Agricultural Sciences –1029.,2019;50(1):343-352 <https://doi.org/10.36103/ijas.v50i1.300>
41. Sellati TJ, Sahay B. Cells of Innate Immunity: Mechanisms of Activation. In: McManus LM, Mitchell RN, editors. Pathobiology of Human Disease. San Diego, CA, USA: Academic Press; 2014. p. 258-274. <https://doi.org/10.1016/B978-0-12-386456-7.01804-9>
42. Kaur S, Bansal Y, Kumar R, Bansal G. A panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. Bioorg Med Chem. 2020;28(5):115327. <https://doi.org/10.1016/j.bmc.2020.115327>
43. Murphey ED, Herndon DN, Sherwood ER. Gamma Interferon does not enhance clearance of *Pseudomonas aeruginosa* but does amplify a proinflammatory response in a murine model of post. Septic Immunosuppression. Infect Immun. 2004;72(12):6892-6901. <https://doi.org/10.1128/IAI.72.12.6892-6901.2004>
44. Akramienė D, Kondrotas A, Didžiapetrienė J, Kėvelaitis E. Effects of  $\beta$ -glucans on the immune system. Medicina. 2007;43(8):597-606. <https://doi.org/10.3390/medicina43080076>
45. Huang J, Wu C, Tang S, Zhou P, Deng J, Zhang Z, et al. Chiral Active  $\beta$ -Glucan Nanoparticles for Synergistic Delivery of Doxorubicin and Immune Potentiation. Int J Nanomedicine. 2020;15:5083-5095. <https://doi.org/10.2147/IJN.S258145>
46. Senoglu N, Yuzbasioglu MF, Aral M, Ezberci M, Belge Kurutas E, Bulbuloglu E, et al., Protective Effects of N-Acetylcysteine and  $\beta$ -Glucan Pretreatment on Oxidative Stress in Cecal Ligation and Puncture Model of Sepsis. J Invest Surg. 2008;21:237-243. <https://doi.org/10.1080/08941930802180136>
47. Sonck E. Immunomodulation of Porcine Leukocytes and Dendritic Cells by (1,3)  $\beta$ -Glucans [Doctoral dissertation]. Ghent University; 2011. <http://hdl.handle.net/1854/LU-1989184>
48. Mahdi NR, Yassein SN, Khalaf JM. Protective effect of cytosine – phosphate-guanosine oligodeoxy nucleotide against experimental mastitis induced by *Cryptococcus neoformans* infection in goats. Iraqi J Vet Med. 2017;40(2):113-123. <https://doi.org/10.30539/iraqijvm.v40i2.122>
49. Touma MM, Jassim HS, Hyyawi SM, Nayyef HJ, Abbas AH. The Role of IFN- $\gamma$  and TNF- $\alpha$  In Experimental Mastitis. Iraqi J Agric Sci. 2021;52(1):121-128. <https://doi.org/10.36103/ijas.v52i1.1242>

## فعالية البيتا جلوكان في علاج التهاب الضرع في الماعز الناجم عن المبيضات البيضاء

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### الخلاصة

ان الغرض من الدراسة الحالية هو تقييم فعالية البيتا جلوكان في علاج التهاب الضرع المتسبب بالمبيضات البيضاء في الماعز. تم استخلاص البيتا جلوكان المستخدم في هذه الدراسة من خميرة المبيضات البيضاء المعزولة من حليب الماعز المصابة بالتهاب الضرع بواسطة طريقة القلوي – الحامض. تم استخدام عشرين من الماعز المرضع (بعمر ٢-٤ سنوات و وزن ٢٥-٣٠ كغم)، ستة عشر منها تم حقنها عبر الضرع ب ١,٢x١٠٧ خميرة/مل، من المبيضات البيضاء الضارية. تم تقسيم الماعز بشكل عشوائي الى خمس مجموعات متساوية بالعدد، حيث كانت المجموعة الاولى كمجموعة سيطرة سالبة (بدون اصابة) والمجموعة الثانية كمجموعة سيطرة موجبة بدون علاج، اما المجموعة الثالثة تم علاجها بالنيستاتين وبجرعة ٢٠٠,٠٠٠ وحدة/شتر/يوم ولسيبعة علاجات. والمجموعة الرابعة عولجت بالبيتا جلوكان وبجرعة ٥ ملغم/مل/شتر/يوم ولثلاث علاجات بين جرعة وأخرى ٤٨ ساعة. اما المجموعة الخامسة عولجت بالبيتا جلوكان فضلاً عن نيستاتين وبنفس الجرع والفترات وبين علاج واخر ٢ ساعة. تم تقييم العلامات السريرية للضرع وجوده الحليب في ضوء استخدام اختبار كاليفورنيا لالتهاب الضرع والعزل الفطري ومستويات IL-6 و IFN- $\gamma$  في المصل بواسطة فحص الاليزا ELISA في الأيام ٣, ٥, ١٠, ٢٠, ٣٠ و ٤٠ يوماً بعد الإصابة. أظهرت النتائج ان العلامات السريرية للضرع في المجموعة الثانية. في حين أظهرت الماعز التي عولجت بالبيتا جلوكان انخفاضاً ملحوظاً في الاعراض و العزل الفطري حيث كان سالباً في يوم ١٥ من التجربة، واما الحيوانات التي عولجت بالبيتا جلوكان زيادة على نيستاتين اختفاء في التهاب الضرع السريري والتحت السريري والعزل الفطري في ٢٥ يوماً من التجربة. وكان انترلوكين ٦- والنترفيرون-٦ (IL-6 and IFN- $\gamma$ ) أعلى مستوى في مجموعة السيطرة الموجبة مقارنة بمجموعة السيطرة السلبية. وبالنسبة لمجموعة البيتا جلوكان فقد ظهر تأثير معنوي اقل من مجموعة السيطرة الموجبة في حين كانت مجموعة النيستاتين ومجموعة البيتا جلوكان و النيستاتين بشكل ملحوظ اقل من مجموعة السيطرة الموجبة وأقل من مجموعة البيتا جلوكان و مجموعة السيطرة السالبة. وتؤكد هذه الدراسة قدرة البيتا جلوكان على علاج التهاب الضرع الناجم عن المبيضات البيضاء في الماعز وكذلك قدرته على خفض مستويات السيتوكينات الالتهابية وكذلك التخلص من الإصابات الفطرية وهذا يشير الى استخدامه كوسيلة علاجية واعدة لعلاج التهاب الضرع الفطري.

الكلمات المفاحية: التهاب الضرع المعزي، التهاب الضرع الفطري، المبيضات البيضاء، بيتا جلوكان، انترلوكين ٦-، نترفيرون-٦