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Original Article

Vaccination Protocol with Purified Mannoproteins Against Invasive Candidiasis

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ABSTRACT

Mannoprotein was extracted and assessed as a promising vaccine against *Candida albicans*. The crude extract was initially identified by Fourier- transform infrared spectroscopy (FTIR) that established the high level of similarity with the mannoprotein profiles, which suggested preliminary identification. After the primary extraction, a purification was done to increase antigenic specificity. The resulting, purified mannoprotein fraction was then produced as a vaccine and immunized into rats in a control experimental protocol. Immunological measurements revealed a moderate, non-significant elevation in concentration of IgG and inflammatory cytokines, including Th1/Th17- associated mediators, before challenge. In spite of not statistically significant of these changes, the current findings indicate immune activation of humoral and cellular immune response. Anyway, after experimental challenge with viable *C. albicans*, a clear decline in IgG antibody titer and inflammatory cytokines was observed in the treated groups by comparing to the control group. This reduction may reflect antigen consumption during active infection, reflecting immune engagement and pathogen-neutralizing activity. The post-challenge decrease may also indicate that the vaccine competent to early pathogen clearance, so it can reduce the need for sustained inflammatory signaling pathway. Generally, our study proves that purified mannoprotein controls immunogenic potential, capable of priming an early immune response before infection. These results support further development of mannoprotein-based vaccines as a promising strategy for prophylactic control of candidiasis. Further studies focusing on long-term immune memory and preventative potency across different infection models are recommended to confirm these preliminary results. The absence of an unvaccinated non- infected control group represents a limitation of our study and may affect the interpretation of immune responses.

Keywords: IgG, Candida albicans, cytokines, Vaccine, Mannoproteins.

Introduction

Candidemia, which represents the most common clinical manifestation of the invasive candidiasis, causes a significant financial burden on the healthcare system. [1, 2]. Epidemical studies have shown that its prevalence is increasing annually. [3], Candida species have become the fourth most common etiologic agent of nosocomial BSIs in the United States and are the most common cause of fungal sepsis in Europe. [4]. With a 30-day mortality rate of 37% following a positive blood culture, candidemia represents a serious public health threat [5]. Immunosuppression, uncontrolled medications of broad-spectrum antibiotics and invasive procedures are closely connected with increased risk of candidemia. Because of the low growth rate of yeasts, the antifungal susceptibility testing and identification of Candida spp. is a time-consuming process that makes it difficult to diagnose and treat early [4]. Nevertheless, timely treatment, both antifungal and supportive interventions are important with respect to patient outcome. Therefore, there is a need to have early empirical care with antifungal drugs using epidemiology to enhance prognosis of patients with fungemia.

In the last 20 years, a number of experimental vaccines against *C. albicans*, which target cell wall proteins, polysaccharide and secreted enzyme components, have been investigated. Nevertheless, a significant number of them are immunogenically ineffective in the protection. Out of potential antigenic targets, mannoproteins have become of particular interest as particularly attractive targets (possess abundant glycoproteins on the fungal cell wall surface). Mannoproteins are presented to the host immune recognition and have some central roles in adhesion, invasion, biofilm formation, and immune evasion [6,7]. Their structure, compositing of a protein backbone heavily glycosylated with mannose residues, makes mannoprotein highly immunogenic and suitable for vaccine development.

Mannoproteins induce both humoral and cellular immune responses. They activate dendritic cells, enhance Th1 and Th17

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polarization, and stimulate cytokines such as IFN-γ and IL-17, which are critical for antifungal defense. At the same time, they evoke antibody production and facilitate opsonophagocytosis by neutrophils and macrophages [8,9]. Such dual immune activation is necessary, as effective antifungal immunity requires both T cell–mediated and antibody-mediated immunity.

The experimental studies also support the possibilities of mannoprotein vaccines. several murine studies report improved survival or decreased fungal burden after mannoprotein-based vaccination after fatal *C. albicans* challenge. When formulated with suitable adjuvants, mannoproteins were able to stimulate long-term memory responses, giving strong protection [10,11]. Moreover, their relatively conserved structure across *Candida* species implies the probability of broad-spectrum protection [12].

Nevertheless, challenges still improving antigen stability, increasing delivery efficiency, and attaining robust immunogenicity, improving antigen stability, increasing delivery efficiency, and attaining robust immunogenicity, particularly in hosts with immune dysfunction. Immunocompromised patients, who are the most susceptible, may display suboptimal vaccine responses. Thus, novel approaches such as nanoparticle encapsulation, liposomal delivery, and conjugation with potent adjuvants are being probed to enhance efficacy, even in hosts with suppressed immunity [13,14]. Advances in nanotechnology and immunomodulatory platforms supply opportunities to overcome these barriers and make mannoproteins vaccines clinically applicable.

Materials and Methods

Extraction of mannoproteins were done according to Wahyudi *et al*. (2024) with some modification, *Candida albicans* M327A strain isolated from Ibn-Gazwan hospital from child infected with oral candidiasis were cultured on SDA medium for (2-5) days then the colonies harvested to obtain 30 gm from 250 ml of media. Water-soluble mannoproteins were isolated from yeast grown in SDA medium (Hi-Media, India) by NaOH (1% (w/v)) lysis of the culture at 100^{o} C for 2h, with cooling and neutralization in 4M HCl. The neutralized extract was then centrifuged at 3000 rpm for 10 min to obtain the supernatant. Thereafter, 200 ml (equivalent to 4 volumes) of absolute ethanol was added and the mannoproteins precipitate. The formed precipitate was then washed with absolute ethanol [15].

Characterization of Mannoprotien by Fourier transform infrared (FTIR) spectrum:

To characterize the functional groups of the bioactive compound within mannoproteins, the functional groups and chemical bonds (pre purification) were detected by analyzing the pellet. The peaks was limited at the range of 400-4000 cm-1 with resolution of 4 cm-1 [16].

Purification by gel filtration chromatography

Sephadex G-150 was made as described in the protocol given by Pharmacia Fine Chemicals Company. A given aliquot of Sephadex G-150 was dispersed in 0.05 M phosphate buffer at pH 7 and heated at 90degC for 5 h to ensure the swelling of the beads, degassed and packed into a glass column (30 x 2 cm). The column was then equilibrated using the same buffer. Mannoprotien extracted from the sample was loaded on to the column. Elution was done at a flow rate of 30 mL of buffer per hour. The absorbance of each fraction collected was measured at 280 nm and protein concentration was determined by the Bradford assay (1979) [17].

Determination of protein concentration

Protein concentration was measured as the method of Bradford (1979) with bovine serum albumin standard curve as follows:

Different concentrations (0, 0.1, 0.2 through 1 mg/mL) were prepared from a stock solution of BSA (1 mg/mL) as indicated in Table (1). Afterward, Coomassie Brilliant blue G -250 dye prepared in (B) was added (2.5 mL), mixed, and stood for 2 min at room temperature. Absorbance at 595 nm was measured; the blank was prepared from 0.45 mL of 0.05 M phosphate buffer pH 7 and 2.5 mL of the dye reagent. A standard calibration curve of concentrations of BSA with corresponding Absorbance 595 nm was plotted (Fig. 1). Protein concentration was determined by adding 0.05mL of the test sample to 0.45mL of phosphate buffer and 2.5mL of Coomassie Brilliant Blue G-250 and bringing it to standstill for 2min at room temperature after which absorbance was measured at 595nm.

Vaccination schedule

Adult female albino rats (Rattus norvegicus), aged 8-10 weeks and weighing 200-250 g were used for the present investigation.

The animals were obtained from the institutional animal house facility and kept under strictly controlled environmental conditions, i.e. a temperature of 22±2 degree Celsius, a relative humidity of 50-60 percent, and a 12-hour light/dark period. Standard laboratory chow and water was provided ad libitum. Mannanoprotein (MNP) was employed as the immunizing antigen. Rats were subcutaneously injected with the vaccine prepared by diluting 50µl of vaccine in 0.1 ml PBS. Injections were administered once weekly for a total period of 21 days (three doses).

Two weeks after the last immunization, rats were challenged with the pathogenic organism (6*10^{5} per 0.1ml) (**viable *Candida albicans* strain**) [18].

At 21 days post-infection, rats were sacrificed under aseptic conditions. Blood samples were collected for serum separation to evaluate (IgG, IL-1β, IL-17, IL-18, and IL-23) levels in all groups.

Statistical analysis

All data were analyzed using the software package (SPSS version 26). Differences between groups were tested using analysis of variance (ANOVA), one way, with post hoc Tukey test. P-value < 0.05 was considered statistically significant. Spearman's rank

correlation coefficient was used to show the relationship between antibodies and cytokines for each group. This non-parametric test was used due to the small size of the sample and abnormal distribution of data.

Results

From repeated culture of *C. albicans*, almost about 3.2 gm. of crude Mannoprotiens were extracted from yeast, it appears with white semi solid viscous product. 2 g of it were dried for FTIR characterization appeared with light brown color, and 1.2 gm. were preserved for further purification by gel filtration lyophilized by freeze drier.

FTIR Spectrum Analysis:

Fourier transform infrared spectroscopy (FTIR), was used to determine the functional groups contained in the sample vaccine. The FTIR spectrum of the isolated mannoprotein revealed characteristic peaks corresponding to both protein and carbohydrate components. The wide absorption band which appears at 3277 cm-1 resembles the O-H and N-H stretching vibrations and indicates the existence of hydroxyl functions inside the mannose residues, as well as amide linkages in the protein constituent. A further analysis of the amide I and amide II bands, which are found at 1621 and 1500 cm-1 respectively, supports the presence of a protein backbone. Finally, absorption peaks detected at 1236, 1110 and 1023 cm-1 are characteristic of C-O-C and C-O stretching modes, and hence reflect glycosidic linkages linking mannose units to each other and the protein matrix. Additional signals in the 856–719 cm^{-1} region support the presence of sugar ring vibrations, confirming the carbohydrate moiety. Overall, the FTIR analysis proved that the isolated compound (figure 1), is consistent with a mannoprotein structure and the FTIR spectrum demonstrates the structural and chemical identity of the extracted mannoprotein with standard compound figure (2).

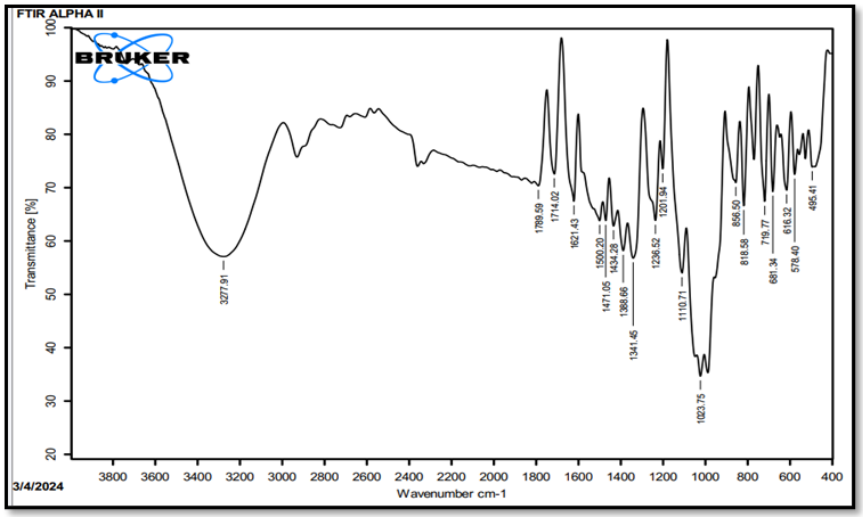

Figure 1: Crude Mannoprotiens peaks of FTIR analysis.

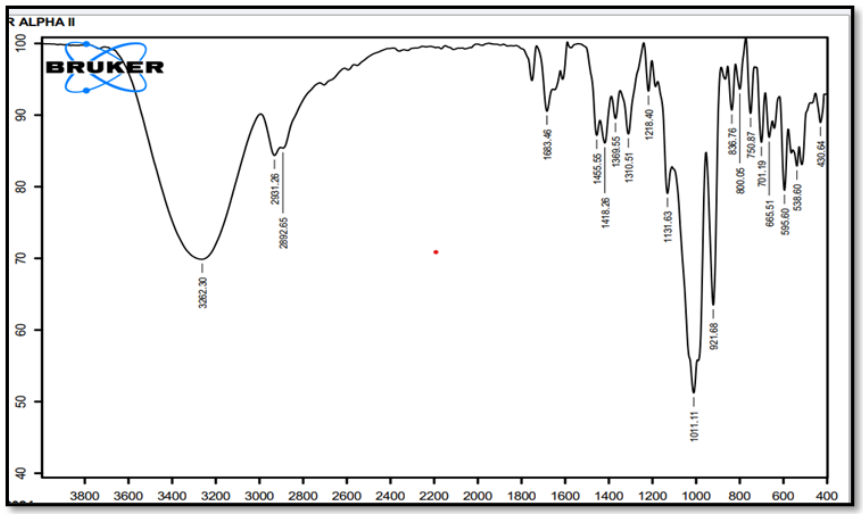

Figure 2: Mannoproteins Standard peaks of FTIR analysis.

Proteins and Sugars Concentrations

Results in figure (2) shows active peaks, fractions from 6-12 shows mannose concentrations, fractions from 6-19 shows protein concentrations in tubes with 5 ml.

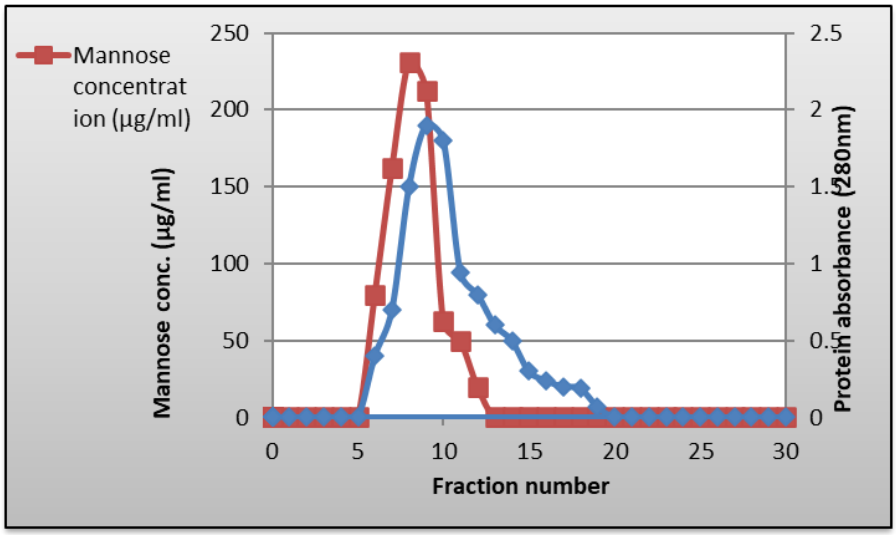

Figure 3: Gel filtration chromatography for mannoprotein purification from Candida albicans with the use of Sephadex-G150 column.

Table 1: Proteins and sugar concentrations in purified Mannoproteins.

Tube no.	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Protein conc.	**0.4**	**0.7**	**1.5**	**1.9**	**1.8**	**0.94**	**0.8**	**0.6**	**0.5**	**0.3**	**0.24**	**0.2**	**0.19**	**0.07**
Mannose conc.	**80**	**162**	**231**	**212**	**62.5**	**50**	**20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

- Counting of viable *Candida* cells

Candida albicans were cultured in Sabouraud Dextrose Broth with 0.5% Tween 20 for 48-72 hours. For the challenge infection the inoculum was adjusted to 6*105 cells/mL. Clinical signs were monitored during 21 days in rats that were vaccine treated. There were no mortalities during this period. Rats of the control group showed less activity and more lethargy than the other groups and their body weight steadily rose until animals were sacrificed. Necropsy of the control animals showed white- to creamy-colonies of *Candida albicans* on several organ surfaces.

- Evaluation of humoral and cellular immune response

The titers of immunoglobulin G (IgG) were determined in the sera collected from the vaccinated and control rats. Statistical analysis have indicated that there is a non-significant increasing in IgG concentration in the group receiving the mannoprotein vaccine (M1: 6.003±1.879) as compared with the control group (C1: 4.450±0.847). Likewise, no statistically significant reduction in IgG levels was recorded in the challenged rats vaccinated with mannoprotein (M2: 5.062±1.386) than in the corresponding control groups (P >= 0.05), as can be seen in Figure 3.

Furthermore, the statistical analysis results summarized in figure (4) revealed that there was non-significant increasing in IL-1β concentration in rats vaccinated with Mannoproteins (40.173±25.532) by comparing with control group (33.243±16.241). While the results after challenging infections showed that there was non-significant reduction in IL-1β titers between challenged rats vaccinated with mannoproteins (57.429±18.626) by comparing with control groups (69.126±38.111).

Also, the present study showed that there was non-significant increasing appeared in concentrations of IL-18 in mannoproteins vaccinated group (152.780±26.603) in contrast with control rat (138.013±27.350). while there was non-significant decreasing in concentrations of IL-18 in vaccinated group with mannoprotein after challenging (135.844±23.204) in compare with non-vaccinated control group (141.335±11.790) (P>0.05), figure (5).

Furthermore, statistical analysis showed a significant increase in IL-17 concentration in the rats vaccinated with the mannoprotein vaccine (10.358± 2.115+) as compared to the control rats (7.128 ±0.952+) with P <0.05.And a non-significant reduction appeared in concentrations of IL-17 in mannoprotein vaccinated group (6.692±2.293) in compare with control group after challenging (8.800± 2.307). (P>0.05), figure (6). Statistical analysis in figure (7) appeared that there was significant increasing in level of IL-23 in rats vaccinated with mannoprotein (45.490±5.401) by

comparing with control group (39.164±3.134) (P≤0.05). After challenging with pathogen, results showed that there was a high significant declining in concentrations in IL-23 in rats vaccinated with mannoprotein (34.976 ±1.047) by comparing with control rats (43.834 ±1.656), (P≤0.01).

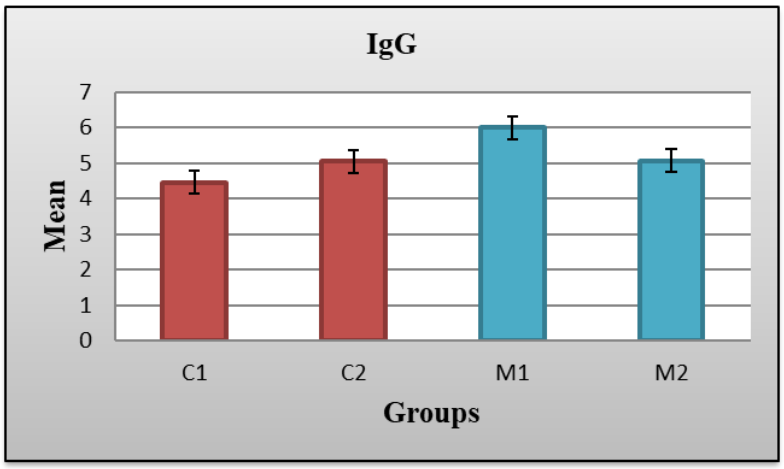

Figure 3: IgG concentration in treated and control group before and after challenge.

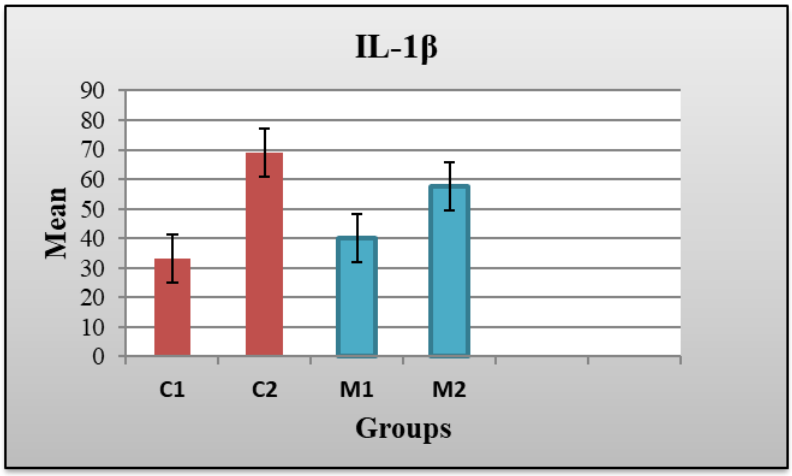

Figure 4: IL-β concentration in treated and control rats before and after challenge.

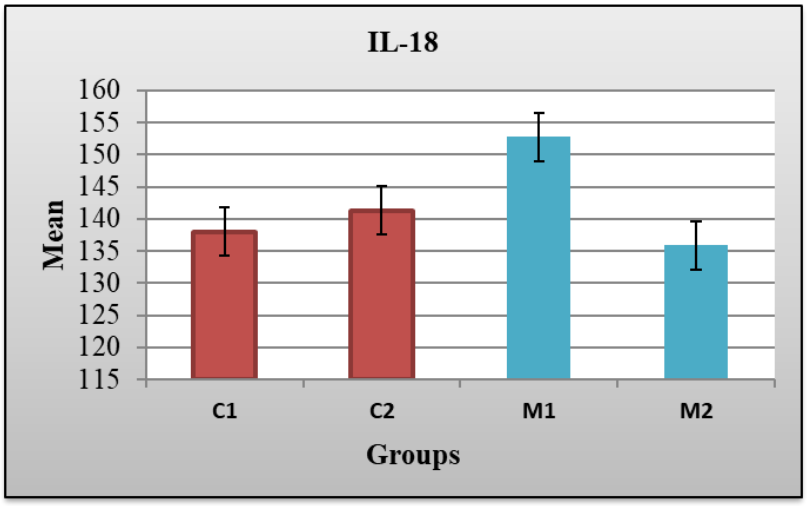

Figure 5: IL-18 concentration in treated and control group before and after challenge.

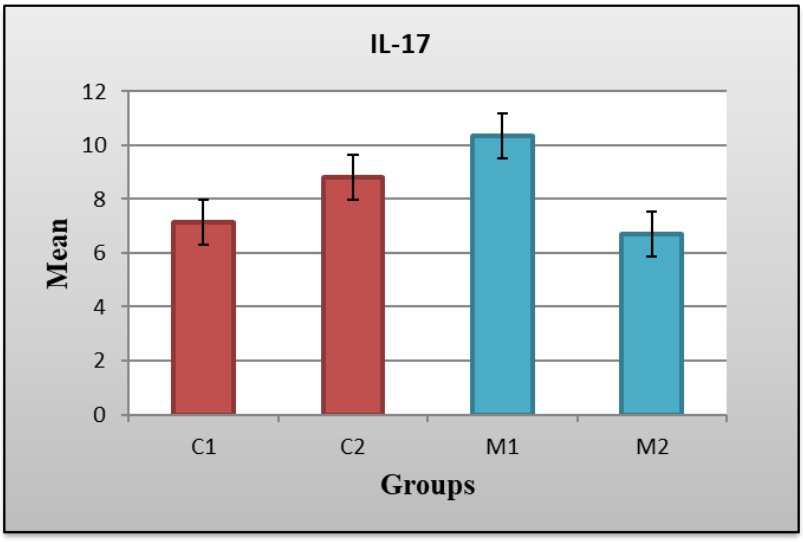

Figure 6: IL-17 concentration in treated and control rat before and after challenge infection.

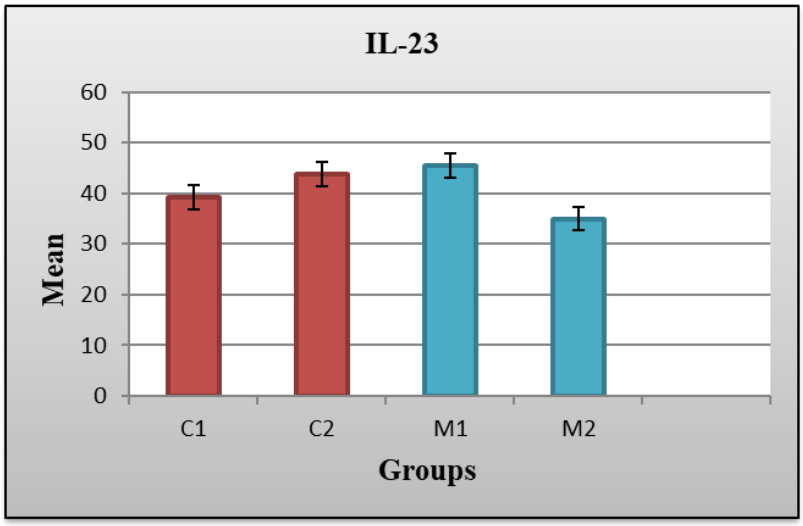

Figure 7: IL-23 concentration in treated and control rat before and after challenge.

Correlation among studied parameters

The interaction between humoral and cellular immune responses is an important in understanding the immunological status of vaccinated rats. Serum immunoglobulin G (IgG) is an important indicator of humoral immunity, whereas pro-inflammatory cytokines such as IL-1β, IL-18, IL-17 and IL-23 are indicators of cellular immune activation and immunological regulation.

Given the small sample size (N=10), the Spearman's rank correlation coefficient for IgG concentrations and the above-described levels of cytokines was used to assess the relationship between both variables in the experimental groups. In the case of mannoproteins treated group (M1), the results indicated no statistically significant correlations between IgG and any of the studied cytokines (all $p > 0.05$). IgG showed weak negative correlations with IL-1β ($r=-0.159$, $p=0.661$), IL-18 ($r=-0.248$, $p=0.489$), IL-17 ($r=-0.214$, $p=0.553$), and IL-23 ($r=-0.484$, $p=0.156$); however, none of these associations reached statistical significance.

Regarding cytokine–cytokine relationships, IL-1β exhibited weak positive correlations with IL-18 ($r=0.146$, $p=0.688$) and IL-17 ($r=0.358$, $p=0.310$), while IL-18 showed a moderate positive correlation with IL-17 ($r = 0.474$, $p = 0.166$) and a moderate positive correlation with IL-23 ($r = 0.573$, $p = 0.084$). Nevertheless, all inter-cytokine correlations were statistically non-significant.

Overall, these findings demonstrate an absence of significant associations between IgG and the evaluated cytokines, as well as among the cytokines themselves, in Group M1.

In control group (C1), the results demonstrated no statistically significant correlations between IgG and any of the studied cytokines, as all p-values were greater than 0.05. A weak negative

correlation was observed between IgG and IL-1β (r=−0.136, p=0.707), while an almost negligible negative correlation was found between IgG and IL-18 (r=−0.003, p=0.993). In addition, IgG showed a weak negative correlation with IL-17 (r=−0.184, p=0.611). Conversely, a weak positive correlation was observed between IgG and IL-23 (r = 0.165, p = 0.650); so, this correlation was statistically non- significant.

Regarding the inter-cytokine relationships within Group C1, no significant correlations were detected. IL-18 was positively correlated with IL-17 at a moderate level (r=0.377, p=0.283), while a moderate negative correlation was noted between IL-17 and IL-23 (r=−0.369, p = 0.294). Nevertheless, none of these correlations reached statistical significance.

In mannoproteins vaccinated group after challenge (M2), correlation analysis revealed statistically significant positive associations between IgG and both IL-17 (r = 0.657, p=0.039) and IL-18 (r=0.658, p = 0.038). These results indicated that higher IgG levels are associated with increased concentration of IL-17 and IL-18 in studied group. No statistically significant correlations were seen between volume IgG concentrations and IL-1β levels (r=0.258, p=0.472) or IL-23 (r=0.408, p=0.242).

Moreover, in the control challenged group (C2), the analysis revealed a significant positive correlation between IgG and IL-18 (r=0.688, p=0.028), indicating that higher IgG levels were associated with increased IL-18 concentrations. In contrast, the correlations between IgG and IL-1β (r=0.449, p=0.193), IL-17 (r=0.356, p= 0.312), and IL-23 (r=−0.172, p = 0.636) were not statistically significant.

Regarding cytokine–cytokine relationships, a strong positive and statistically significant correlation was observed between IL-1β and IL-18 (r=0.798, p=0.006). No significant associations were detected between IL-17 and the other cytokines, including IL-1β (r=−0.078, p=0.831), IL-18 (r=0.060, p=0.870), or IL-23 (r = 0.058, p = 0.873). Similarly, IL-23 showed no significant correlation with the remaining cytokines.

Overall, these results demonstrate that within Group C2, IgG is significantly associated with IL-18, and a strong interrelationship exists between IL-1β and IL-18, while the remaining correlations were weak and not statistically significant.

Discussion

Candida albicans cell wall consist of polysaccharides, protein and lipids, the proteins conjugated with mannan in the form mannoproteins. Variable extraction methods have been used to extract mannoproteins from yeasts cell wall; e.g alkali acidic method, in the current study we used the common methods to extract and purify mannoproteins from *C. albicans.* The successful extraction of a viscous mannoproteins fraction using acid–base treatment (HCl/NaOH) is consistent with recent reports describing how chemical disruption of yeast cell walls frees mannoproteins efficiently. Acidic-alkaline treatments are known to solubilize β-glucans and glycoprotein bonds, though conditions strongly influence yield, purity, and the preservation of functional domains. The viscosity observed in the present study agrees with the release of high-molecular-weight, heavily glycosylated mannoproteins, a property emphasized in contemporary extraction studies [19].

The FTIR spectrum of the extracted compound revealed the characteristic absorption bands of mannoproteins. Prominent peaks corresponding to polysaccharide vibrations (C–O–C and C–O stretching between 1000–1150 cm^{-1}) together with amide bands (Amide I at ~1650 cm^{-1} and Amide II at ~1540 cm^{-1}) confirmed the glycoprotein nature of the isolate. These spectral features are in strong agreement with previous studies that used FTIR to identify mannoproteins from *Saccharomyces cerevisiae* [20].

In the current study, the non-significant elevation of Immunoglobulin G antibody in groups vaccinated with mannoproteins vaccine indicates that the vaccines may act immune stimulator for adaptive immunity because its efficiency in activation B lymphocytes to produce specific antibodies. While after challenge infection, the decline of IgG concentration within the vaccinated group compared to their pre-challenge levels can be related to several immunological processes occurring simultaneously. The most likely is the hasty degradation of antibodies by the development of antigen-antibody complexes, and these complexes are eliminated through the reticuloendothelial system. Simultaneously, antibodies produced by the vaccines might have moved out of the blood to sites of infection where they take part in opsonization and neutralization of the pathogen, which causes them to be undetectable in serum. Another contributing factor is that of post-challenge isotype switching or a switch to antibody subclasses that are not best detected by the ELISA system used. Finally, immune regulatory pathways and antigenic modulation by Candida-such as masking or remodeling of cell-wall epitopes could reduce detectable antibody binding Together, all these mechanisms demonstrate that the declining in circulating antibodies after the challenge is probably an active immune process and use of antibodies in clearing pathogens, and not the loss of immune protection [21,22,23].

The non-significant elevation of IL-1β levels in the mannoprotein-vaccinated group compared with the control indicates that the vaccine formulation activated innate immune mechanisms. Mannoproteins derived from *Candida albicans* are known to be potent pathogen-associated molecular patterns (PAMPs) recognized mainly by dectin-1 and Toll-like receptors on macrophages and dendritic cells, leading to NF-κB activation and pro-IL-1β transcription. The inclusion of Freund's adjuvant, which contains mineral oil and *Mycobacterium*-derived components, further amplifies this response by stimulating inflammasome assembly and enhancing cytokine secretion, including IL-1β. This explains the higher baseline concentrations in the treated group compared to the control before fungal challenge [24,25].

Following *Candida* infection, IL-1β levels in the vaccinated group increased relative to their pre-challenge values but remained lower than those in the challenged control group. This

pattern suggests that non-significant result indicated no reliable activation of innate immune system, thereby reducing the duration and magnitude of inflammatory signaling required for control of infection. In contrast, unvaccinated controls exhibited uncontrolled fungal proliferation, resulting in sustained inflammasome activation and exaggerated IL-1β production. Moreover, Freund's adjuvant is known to induce both effector and regulatory cytokines; hence, the moderate IL-1β response post-challenge in the vaccinated group may reflect faster activation of regulatory mediators such as IL-1 receptor antagonist and IL-10, limiting excessive inflammation while maintaining protection. Collectively, these results indicate that the mannoprotein-Freund's formulation enhances the early IL-1β-dependent immune response yet ensures a controlled inflammatory environment upon subsequent exposure to *Candida albicans*, consistent with balanced and effective vaccine-induced immunity or it may be due to biological variation rather than vaccination, [26,27,28].

The present study indicated a significant increasing in serum IL-18 titers in the groups vaccinated with the mannoprotein-based vaccine compared to the non-vaccinated control group. Such increase can be explained as a direct effect of the vaccine which stimulate the activation of the innate immune system, especially the induction of the activities of macrophages and dendritic cells., which are known to produce IL-18 upon recognition of fungal mannans by pattern recognition receptors (PRRs) e.g., TLR4 and dectin-2. Mannoproteins from Candida species possess potent immunostimulatory characters can induce Th1/Th17 cytokine production, including IL-18, that functions synergistically with IL-12 to enhance IFN-γ secretion and promote cell-mediated immunity. Thus, the finding of the increasing of IL-18 in vaccinated groups might indicate the adequate priming of the immune system and the creation of a pro-inflammatory protective environment, preparing the host to execute fungal clearance in a more efficient manner in case of a challenge [29].

After Candida challenge, serum IL-18 levels of the vaccinated group non-singnificantly decreased in comparison to the control-infected group, this result suggests an immunomodulatory effect rather than immune suppression. IL-18 is a key pro-inflammatory cytokine related with early innate immune activation. The reduction may indicate that prior vaccination primed the immune system, enabling a more controlled response to infection and decreasing the need for excessive inflammasome-mediated cytokine release. The absence of statistical significance may be due to limited sample size or the modest regulatory nature of the vaccine-induced response.

Conversely, in response to the fungal challenge, the control group with no prior immunological preparedness responded with a delayed, excessive inflammatory response in response to the challenge, as denoted by high IL-18 and the host attempted to compensate by a poor initial response to control the fungi. Thus, the reduction of IL-18 in the vaccinated groups after infection may be viewed not as the inadequate immune response, but as the evidence of the controlled and regulated inflammation, which are available to the efficient adaptive recollection response that reduces immunopathology and provides fungal clearance [30].

Besides its effects as an early inflammatory mediator, IL-18 is a significant cytokine in the coordination of Th1-polarized immune responses, interacting synergistically with IL-12 resulting in the strong induction of IFN- 7 by NK cells and $CD4^+$ T helper cells. This cytokine pathway is also found to be a very important mechanism in the host defense mechanism against Candida infections because IFN- 7 is the one which causes the macrophages and neutrophils to increase their fungicidal abilities as well as increase the growth of protective cellular immunity as opposed to over stimulating the inflammatory response [31]. The early increase of IL-18 before challenge in vaccinated groups was probably the cause of rapid NK cells activation, thereby allowing them to provide an early cytotoxic and cytokine-mediated inhibition of fungal growth, thereby sparing them the burden of prolonged systemic inflammation during an infection. According to van de Veerdonk *et al*. (2011), pre-activation of NK cells not only restrain the proliferation of fungi, but also regulates the activity of inflammasomes that will excessively release IL-18 with the progression of infection. Thus, the immunological pattern that manifests itself in the vaccinated animals is associated with a regulated Th1-biased reaction where IL-18 acts as an immunomodulatory intermediary between innate sensing and adaptive memory formation, which is a characteristic of an effective vaccination-mediated defense [32].

The statistically significant IL-17 levels among the rats vaccinated against the mannoprotein-based vaccine versus the control group are statistically significant ($P<0.05$) and this demonstrates that the vaccine was able to stimulate the Th17 axis through vaccination before the exposure to the pathogen. Mannoproteins are highly concentrated in fungal mannan fragments, which bind to C-type lectin receptors like dectin-2 and mannose receptor causing the activation of the dendritic cells and macrophages. The antigen presenting cells then secrete IL-23 that is a major cytokine that stabilizes and increases the population of Th17 cells which eventually increases the release of IL-17. This mechanistic cascade (PRR engagement → IL-23 induction → Th17 differentiation → IL-17 production) is well-recognized as a critical pathway in antifungal host defense and supports the pre-challenge elevation of IL-17 observed in the vaccinated group [33,34].

Interestingly, after Candida challenge, there was no statistically significant increment in IL-17 between the vaccinated and control group ($P>0.05$) although it was initially high before infection. This immune response indicates that vaccinated animals were capable of regulating the multiplication of pathogens more effectively, which decreases the fungal load and consequently the secondary surge of IL-17 is not so high. Conversely, control animals that did not have pre-existing immune priming, were highly likely to have a delayed but exaggerated Th17 response as a compensatory effect due to uncontrolled fungal growth. This control immune response is consistent with the literature that explains how this kind of immunological preparedness can result in a quick and limited inflammation, and thus stopping the

excessive generation of proinflammatory cytokines like IL-17 when an active infection occurs [35].

The elevated IL-23 levels observed in vaccinated animals prior to challenge indicate effective immune priming and activation of antigen-presenting cells. After challenge, the reduced IL-23 levels in the vaccinated group compared to controls suggest a regulated immune response, where prior immunization diminished the need for sustained Th17-supporting cytokine production. In contrast, the non-vaccinated control group exhibited higher IL-23 levels as part of a delayed and compensatory inflammatory response. These findings support the immunomodulatory role of vaccination rather than immune suppression.

Together, these results focus on the distinct immunomodulatory roles of IL-17 and IL-23 after mannoprotein vaccination: IL-23 acts as an upstream regulatory cytokine maintaining Th17 readiness, while IL-17 reflects functional effector activation that may not require further amplification if pathogen control is achieved early, as seen in vaccinated animals.

In the control group (C1), no statistically significant correlations were observed between IgG and any of the measured cytokines. This finding is expected, as the animals in this group were neither immunized nor exposed to an immune challenge, reflecting a basal immune state. Under such conditions, IgG levels remain relatively stable and are not driven by inflammatory cytokine activity.

The weak and non-significant correlations detected between IgG and pro-inflammatory cytokines (IL-1β, IL-18, IL-17, and IL-23) likely represent normal biological variability rather than functional immune interactions. Similarly, although moderate correlations were observed among certain cytokines, these associations did not reach statistical significance, indicating the absence of coordinated inflammatory signaling. Overall, these results confirm that the immune system in the control group remains in homeostasis, providing a valid baseline for comparison with immunized or challenged groups [36].

In the mannoprotein-vaccinated group (M1), no statistically significant correlations were observed between IgG and the assessed cytokines. Weak negative associations were detected, suggesting that IgG production was not strongly influenced by cytokine activity in the absence of antigenic stimulation.

Inter-cytokine analysis revealed some weak to moderate positive correlations, particularly between IL-18 and IL-23, and IL-18 and IL-17, yet none reached statistical significance. These patterns indicate mild immune priming and partial activation of regulatory inflammatory pathways. Overall, the findings suggest that mannoprotein vaccination alone primes the immune system, enhancing readiness without inducing a fully coordinated humoral or inflammatory response. This explains the immunomodulatory nature of mannoproteins, which prepare the immune system for potential challenges instead of eliciting a strong response in the absence of an antigen [37].

Within the mannoprotein vaccinated group which was challenged with infection (M2), Spearman correlation analysis showed a significant positive correlation between IgG and IL 17 suggesting that the immunization succeeded in priming the immune system to produce a Th17 mediated response. This suggests that upon challenge, humoral immunity (IgG production) was coordinated with IL-17-mediated cellular responses. In contrast, correlations between IgG and other cytokines (IL-1β, IL-18, IL-23), as well as inter-cytokine correlations, were weak and not statistically significant. These findings indicate that the vaccine induced a targeted and modest immune activation, sufficient to engage specific pathways without triggering a broad or strong inflammatory respons, [38].

In the non-vaccinated control group (C2) challenged with the pathogen, IgG levels showed a statistically significant positive correlation with IL-18 indicating that higher antibody levels were associated with increased IL-18 concentrations. This suggests that the infection alone elicited a partial humoral response linked to Th1-type cytokine activity. Correlations between IgG and other cytokines (IL-1β, IL-17, IL-23) were not significant, reflecting that the unprimed immune system did not generate a coordinated or broad response. Notably, IL-1β and IL-18 displayed a strong positive correlation, indicating activation of natural inflammatory pathways in response to the pathogen. However, the lack of significant associations with other cytokines suggests limited immune network engagement, [39,40,41].

Conclusion

The current study provides promising evidence that purified mannoprotein extracted from *Candida albicans* can function as an effective immunogenic candidate for vaccine development. The significant increasing in antibody levels and pro-inflammatory cytokines before infection challenge indicates that the antigen moderately activated both humoral and cellular immunity. These post challenge changes in these immune parameters are probably indicative of active immune responses in clearance of the pathogen and not immune suppression, indicating that the immune response induced was functional and it may be protective. These results suggest that mannoprotein does not simply initiate an immune response but can also help to effectively regulate inflammatory responses during active infection to avoid superfluous immune-mediated tissue responses.

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Conflict of interest

Authors declare that there is no financial interests that may affect their integrity regarding this project.

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