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AdhO36 Liposomes from *Salmonella* Typhi in Combination With β-Glucan Immuno-adjuvant From *Candida albicans* Cell Wall as Oral Vaccine Against Typhoid Fever in Mice Model

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Abstract

BACKGROUND: The development of an oral typhoid fever vaccine need more effective and having high-efficacy in preventing typhoid fever. The use of liposomes as a vaccine vehicle can be formulated to target a specific location or trigger the release of antigens on its target. β-Glucan derived from *Candida albicans* cell wall as immunoadjuvant can strengthen the immune response and increases the protection against *Salmonella* Typhi bacterial invasion.

AIM: This study aimed to determine the immune response in typhoid fever mice by administering a combination of AdhO36 *S.* Typhi liposome vaccine with β-Glucan and determine the protectivity to inhibit bacterial colonization in typhoid fever.

METHODS: Mice were divided into five groups include negative and positive control also treatment group. IL-12 was evaluated after 4-h immunization while the other (was IL-12, IL-10, Th1 (IL-2), Th2 (IL-4), and the protective test against bacterial invasion) evaluated after 96-h.

RESULTS: IL-12 level in the combination of β-Glucan and AdhO36 groups showed significantly lower than infected groups ($p = 0.034$), whereas IL-10 level significantly increase ($p = 0.0009$). The percentage of Th-1 (IL-2) cells significantly lower than infected groups ($p = 0.000$), this also happened on the percentage of Th-2 (IL-4) cells that significantly lower than infected groups ($p = 0.018$). The protective test toward bacterial invasion showed no bacterial colonization in all tissues intestine, liver, spleen, and mesenteric lymph node.

CONCLUSION: The administration of a combination of liposome containing β-Glucan from *C. albicans* and AdhO36 *S. Typhi* has a potential effect on cellular and humoral immune response.

Introduction

As it has high morbidity, typhoid fever is an infectious disease that still becomes a health problem, especially in developing countries [1], [2]. Since the mortality is still high, the concern of controlling typhoid fever should be more aggressively through prevention [3]. Preventive measures by administering vaccines including Ty21a oral vaccine and ViCPS vaccine (Vi capsular polysaccharide) given parenterally have approximately between 51% and 67% efficacy, and in Indonesia, the use of enteric capsules dosage form has only 43% efficacy [4], [5]. Considering the protective power obtained from various known types of vaccines are still relatively low, the development of vaccines for controlling typhoid disease is highly needed. Vaccine development efforts are needed, particularly regarding proteins that play a role in bacterial immunity and virulence, namely, adhesin protein. *Salmonella* Typhi does not only have a fimbria adhesin protein called AdhF36 which plays a role in the attachment but also has an afimbrial adhesin protein called AdhO36 (afimbrial adhesin) which mediates a stronger attachment in host

cells [6], [7]. Adhesin protein mediates the attachment of bacterial cells to host cells, so it is a major virulence factor for bacteria. The present invention wishes to develop the potential of the AdhO36 adhesion protein further and combine it with the β-Glucan immunoadjuvant in oral dosage form since it has not been developed yet.

Physicochemical characteristics of active pharmaceutical ingredients or drug compounds in dosage forms or drugs and usage routes are critical determinants of *in vivo* performance, safety, and efficacy of medicinal products [8]. The drug's properties and dosage forms are carefully designed and tested to produce the desired therapeutic response in patients. The importance of drug compounds and drug formulations is in the absorption and distribution of drugs to the site of action. The characteristics of drug ingredients or compounds determine the formulation, drug route, and bioavailability [9]. The dynamic stages of drug sequences that begin from entering through certain usage routes, drug release, and dissolution affect the absorption of the drug. Vaccine vehicle (VV) is used to determine the release and dissolution of the drug into the systemic circulation to the drug in the tissue results in pharmacological or clinical effects. The phase of developing VV by the oral route is needed primarily to prevent antigen degradation and increases the rate of absorption across the biological membrane, thereby increasing its bioavailability and therapeutic index [10], [11]. In this invention, the liposomal VV formulation is designed because liposome can be designed to target a specific location or trigger the antigen release on its target. The effectiveness of liposomal formulations depends on various physicochemical factors such as vesicle size, surface loading, bilayer composition, the presence of coatings, route of administration, use of adjuvants, the efficiency of encapsulation, and the composition of the lipids used [12], [13], [14].

In addition to the factor of increasing the effectiveness of vaccines by oral VV liposome, it is possible to manipulate by adding immunoadjuvant material that can strengthen the cellular immune system. Nowadays, there are many ingredients derived from nature that is potential as immunoadjuvant, one of which is β-Glucan that is a component of polysaccharides and is proven to be a potential immunoadjuvant [15]. β-Glucan is considered a stimulator of cellular immunity that activates macrophages. β-Glucan derived from *Candida albicans* cell walls can inhibit the colonization of *Salmonella* Typhimurium by potentiation in cellular immunity, namely, an increase in $CD4^+$ and $CD8^+$ levels [16], [17]. However, so far, there has no invention that tests whether β-Glucan administration in individuals who have been immunized with liposomal AdhO36 formulation and given orally can increase the immune response.

In silico approach result has shown the potential effect of AdhO36 and beta-glucan combinations enhanced cellular immunity against *S.* Typhi infection [18]. The proposed invention on the use of a liposomal VV that is cationic in nature will provide a better delivery of the AdhO36 antigen system to the target cell, and immunoadjuvant administration can improve cellular and humoral immune responses so that the vaccine is more effective[19], [20].

Liposomes offer advantages as vaccine carriers compared to immuno-stimulating complex because they are able to increase the absorption of antigens in the intestinal area, as well as having good encapsulation efficiency values for hydrophilic and lipophilic antigens. AdhO36 antigens are hydrophilic, while β-glucan is a lipophilic immunoadjuvant [21], [22].

Materials and Methods

Culture and isolation of β-glucans from C. albicans

In this invention, *C. albicans* was obtained from vaginal clinical specimens and cultured in the sabouraud dextrose agar medium and incubated at 37°C for 18–24 h. Gram-staining and observation were done under a microscope at ×1000, and germ tube tests were carried out to identify *C. albicans*. *C. albicans* culture obtained was soft, and creamy and had a special yeast odor. Microscopic analysis was performed by observing under a microscope at ×1000 to ensure that *C. albicans* was cultured correctly. Gram-staining and germ test tube culture of *C. albicans* were also performed and observed under ×1000.

C. albicans culture was harvested and collected by centrifugation at 3000× *g* with phosphate-buffered saline (PBS) pH 7.4. Cell pellets were collected and washed 5 times with lysis buffer (10 mM Tris-HCl combined with 1 mM phenyl-methyl-sulfonyl fluoride) and centrifuged at 3000× *g* for 10 min. Sediments were collected and suspended with lysis buffer at 4°C, and then mechanically lysed with 1-mm glass beads using Omni Mixer Homogenizer at 6000× *g* for 10 min. The lysed cells were separated by centrifugation at 3000× *g* for 10 min. The precipitate obtained was cell wall fraction [23].

Isolation of AdhO36 protein

S. Typhi was isolated from Saiful Anwar Public Hospital's patient, Gram-negative and rod-shaped staining was identified. Identified *S.* Typhi bacteria were reproduced on the McConkey medium and incubated at 37°C for 18–24 h. The culture from the McConkey medium was then transferred to the biphasic medium consisting of LB liquid medium and TCG sloping agar medium, incubated at 37°C for 24 h. Bacteria are harvested and used for the isolation of the AdhO36 protein. The liquid

culture from the biphasic medium was transferred into a 100 cc centrifuge tube, TCA was added so that the concentration was 3%, then centrifuged with PBS solution pH 7.4 sufficiently, then carried out the separation of the fimbria fraction using a modified fili cutting tool at 4°C, rotated at a temperature of 4°C 12,000 rpm for 15 min. The filtrate was separated (containing the fimbria fraction), while the sediment was suspended with sufficient PBS, then the fimbria was cut again. This process is repeated 5 times. The cell fraction that has been separated from the fimbria fraction is suspended with sufficient PBS solution, and then a CHAPS detergent is added with a concentration of 5%. The mixture was vortexed for 5 min, and then centrifuged at 4°C 12,000 rpm for 15 min. The filtrate was taken (coarse Omp fraction), dialysis was performed using PBS dialysate in SDS-PAGE electrophoresis. Electrogram tape at a position of about 36 kDa is cut and collected. Electroelution and dialysis were then applied to the gel pieces using PBS solution so that the pure protein AdhO36 was obtained [23]

VV formulation

Liposomal preparation was carried out using the thin-film hydration method based on the liposomal formula with the ovalbumin antigen model [24]. In the manufacturing process, DDA, soy phosphatidyl-choline (SPC), and cholesterol were dissolved in 5 mL methanol. The lipid phase was the same for all formulas with a percentage of weight to the final volume of the preparation as follows: DDAB 0.025 g; SPC 0.091 g, and cholesterol 0.005 g. Then, the organic methanol solvent was removed using a rotary evaporator for 45–60 min at 45–55°C until a thin layer of lipid phase was formed on the wall of the round bottom flask. To form liposome, the thin film layer was hydrated with a solution of active ingredient β-Glucan or AdhO36. The dose of β-glucan was 300 μg, and the dose of AdhO36 100 μg.

In vivo test

The selected test animals were adult male mice aged 8 weeks weighing 20–30 g. There were five test groups, consisting of five mice in each group. The first group (A) negative control of uninfected mice, the second group (B) was positive control of infected mice with typhoid fever, the third group (C) was liposomal beta-glucan treatment group, the fourth group (D) was liposomal AdhO36 treatment group, and the fifth group (E) was combination of liposomal AdhO36 and liposomal beta-glucan combination treatment group. The positive and negative control groups received VV liposomal blank 3 times every 10 days. The beta-glucan treatment group received 300 μg of beta-glucan by oral route 3 times every 10 days. As much as 300 mL 10^8 cells/mL of *Salmonella* Typhimurium were given twice in 2-day intervals [23], [25], [26]. Each group then evaluated using ELISA test 4 h after the last immunization to measure IL-12 level (Legend Max, USA, Cat. No. 433607). After 96 h after last immunization, IL-10 level (Legend Max, USA, Cat. No. 431417) of all groups was tested using ELISA method and further evaluated Th1 (IL-2) and Th2 (IL-4) level using flow cytometry. Colonization of bacteria tested in some organs such as intestine, liver, spleen, and mesenteric lymph node (MLN) [25]. The percentage of IL-2 (Th1) and IL-10 (Th2) was measured using intracellular staining of flow cytometry [27]. Briefly, the spleen tissues were isolated and single cell suspensions were prepared. The tissue was passed through a nylon mesh (70 μm pore size). For intracellular cytokine measurement, spleen cells (1×10^6) were stimulated for 5 h with PMA (1 μg/mL, Sigma-Aldrich) and ionomycin (50 μg/mL, BD Biosciences) in the presence of monensin (0.1 mg/mL, Sigma-Aldrich) and incubated in a 37°C and 5% CO_2. Spleenic cells were washed with PBS and surface-labeled with anti-CD4–FITC (Biolegend, Uithoorn, Netherlands). Cells were fixed and permeabilized (Cytofix/Cytoperm, BD Biosciences) and stained with anti-IL-2-PE (Biolegend, Uithoorn, Netherlands, cat No. 503808) and anti-IL-4-PE (Biolegend, Uithoorn, Netherlands, cat. No. 504104). After fixation, three-color cytometry was done using a FACSCalibur flow cytometer. A minimum of 10,000 cell-gate events were acquired and analyzed with CELL Quest (Becton Dickinson) software.

This study has obtained the Ethical Clearance from University of Muhammadiyah Malang Indonesia, as stated through decree No.E.5.a/136/KEPK-UMM/VII/2019.

Results

The combination of β-glucan and AdhO36 decrease IL-12 levels

IL-12 levels were examined from plasma examination material using the ELISA method, and

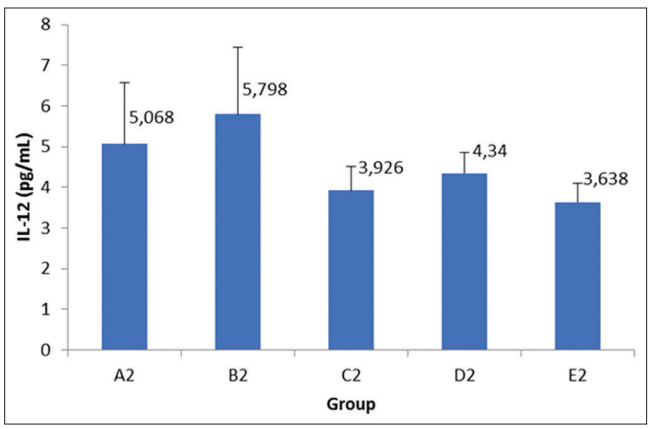

Figure 1: The combination of β-Glucan and AdhO36 decrease IL-12 levels (A2: Uninfected control, B2: Infected control, C2: Liposomal β-Glucan, D2: Liposomal AdhO36, E2: Liposomal β-Glucan-AdhO36)

the absorbance data were converted to levels. From Figure 1 and the ANOVA analysis results in p = 0.030, there were significant differences in IL-12 levels in the treatment of each group. From Tukey HSD test, it was found that in the healthy Group (A) and the infected Group (B), there were no significant differences but there was a tendency to be higher. This result shows that mice were not able to increase cellular immune response. Same result happened on infected Group (B), treatment Group C (β-Glucan), and the treatment Group D (AdhO36) that there were no significant differences. While result of Group E (combination β-Glucan + AdhO36), there was a significant difference compared to the infected control group (p = 0.034).

The combination of β-glucan and AdhO36 increase IL-10 levels

IL-10 is a type 2 cytokine that stimulates humoral immunity by stimulating the development and activation of mast cells and eosinophils, differentiation of B cells to secrete antibodies and switching immunoglobulin B cells to IgE. IL-10 is a very important cytokine because it inhibits macrophage activation, T cell proliferation, and pro-inflammatory cytokine production (Elenkov IJ, Chrousos GP and Wilder RL, 2014). IL-10 parameters in the research results obtained that in treatment E (β-Glucan + AdhO36), the average IL-10 level was the highest compared to other treatments and statistically significant.

IL-10 levels were measured using a plasma examination material in the 96-h group using the ELISA method, and the results that were absorbance were converted to levels. ANOVA analysis results can be seen in the Group (E) combination of β-glucan and AdhO36 liposomes compared to the infected group (p = 0.0009) (Figure 2).

The combination of β-glucan and AdhO36 decrease the percentage of Th-1 (IL-2) cells

The percentage of Th-1 (IL-2) cells was examined from spleen examination material in the 96-h group using the flow cytometry method, and the data analyzed were the percentage of Th-1 (IL-2) cell (UR% Gated). From Figure 3 and ANOVA analysis results, it can be seen that the treatment in all treatment groups and E (β-Glucan + AdhO36) results in a significant difference to (B) infected group (p = 0.000).

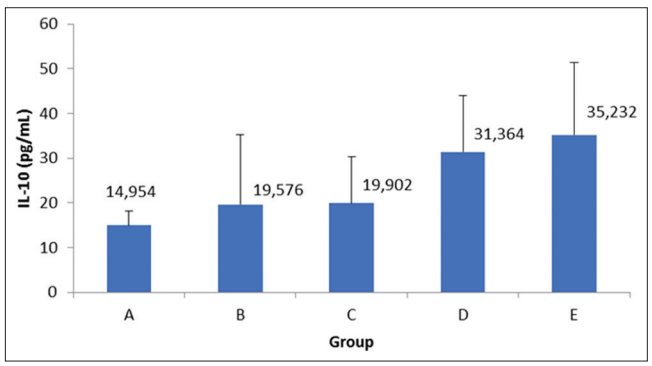

Figure 2: Combination of β-Glucan and AdhO36 Increase IL-10 levels. (A) uninfected control (B) infected control (C) Liposomal β-Glucan (D) Liposomal AdhO36 (E) Liposomal β-Glucan-AdhO36

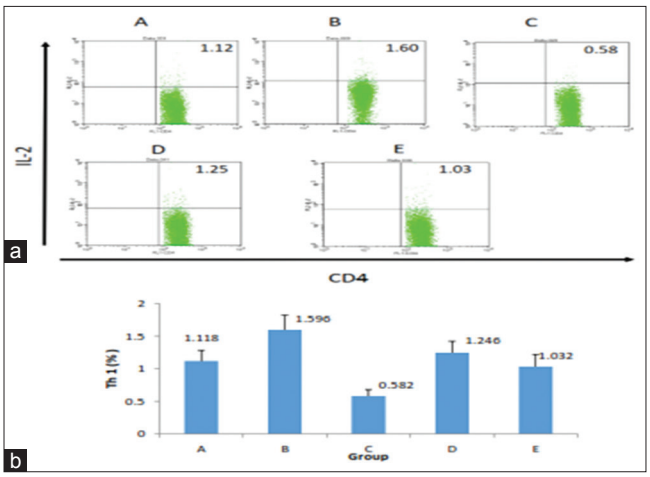

Figure 3: Combination of β-Glucan and AdhO36 decrease the percentage of Th-1 (IL-2) cells. (a) Result the percentage of Th-1 (IL-2) cells were examinated using flow cytometry (b). Histogram ANOVA analysis results. (A) uninfected control (B) infected control (C) Liposomal β-Glucan (D) Liposomal AdhO36 (E) Liposomal β-Glucan-AdhO36

The combination of β-glucan and AdhO36 decrease the percentage of Th-2 (IL-4) cells

The percentage of Th-2 (IL-4) cells were examined from the spleen examination material in the

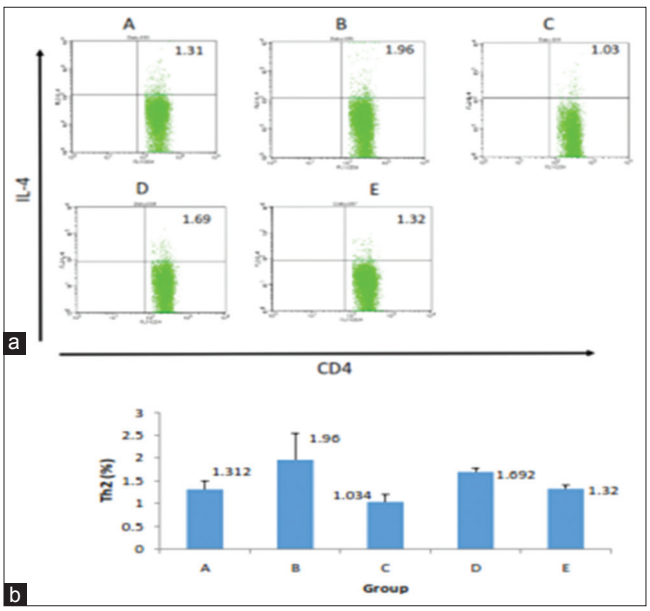

Figure 4: Combination of β-Glucan and AdhO36 decrease the percentage of Th-2 (IL-4) cells. (a) Result the percentage of Th-2 (IL-4) cells were examinated using flow cytometry (b). Histogram ANOVA analysis results. (A) uninfected control (B) infected control (C) Liposomal β-Glucan (D) Liposomal AdhO36 (E) Liposomal β-Glucan-AdhO36

96-h group using the flow cytometry method, and the data analyzed were percentage of Th-2 (IL-4) cell (UR% Gated). From Figure 4 and ANOVA analysis results, it can be seen that in the treatment Group E (β-Glucan + AdhO36), there is a significant difference to infected group ($p = 0.018$).

The combination of β-glucan and AdhO36 is protective to bacterial invasion in all tissues (intestine, liver, spleen and MLN)

The samples used were obtained from organs (intestine, liver, spleen, and MLN) of mice which were immunized according to treatment 3 times with an interval of 10 days and infected twice with a 2-day interval with 10^8 cells/mL *S*. Typhimurium as much as 300 μL after 96 h of organ surgery. The parameters used to measure the effectiveness of liposomal β-Glucan, liposomal AdhO36, and combination treatment of β-Glucan and AdhO36 liposomal are colonization count bacteria that are still alive in the intestine (U), liver (H), spleen (L), and MLN: Mesenteric Lymphoid Node (M) (Figure 5).

Discussion

IL-12 is produced by dendritic cells (DC), macrophages, neutrophils, microglia cells, and a small number of B cells. IL-12 is a cytokine that initiates cellular immune responses associated with inhibition of bacterial invasion. IL-12 is produced as a response to intracellular microbe infection. IL-12 plays an important role in the control of infections by intracellular bacteria such as Salmonella sp. IL-12 plays a protective role in host-defense against *Salmonella* sp. Secretion of IL-12 increases markedly after *Salmonella* sp. infection [28]. IL-12 will induce T-lymphocytes and NK cells to produce INF-γ. IFN- γ is produced by Th1 cells and promote cell-mediated immunity, which is essential for the response against intracellular pathogens. IL-12 is the main cytokine that regulates Th1 differentiation

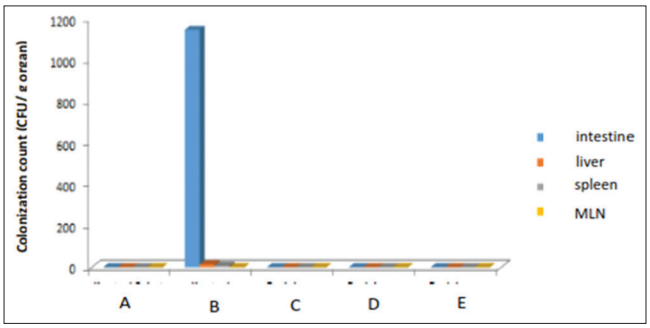

Figure 5: The combination of β-Glucan and AdhO36 is protective to bacterial invasion in all tissues (intestine, liver, spleen, and MLN). (A) uninfected control (B) infected control (C) Liposomal β-Glucan (D) Liposomal AdhO36 (E) Liposomal β-Glucan-AdhO36

and has a number of important actions that serve to promote cell-mediated immunity. From the results of the study (Figure 1), the average IL-12 level in Group B (infected) was 5.798 (pg/mL), which is higher than Group A (healthy). This shows that IL-12 concentrations increase because IL-12 is produced by macrophages and infected DC; IL-12 is produced in response to various microbial stimuli including LPS, infection by intracellular bacterial and viral infections. The IL-12 cytokine is the main cytokine that activates STAT4, which will induce Th1 cellular responses. From the results of the study, it was found that IL-12 level in the Group E (β-Glucan and AdhO36) showed the lowest result compared to other treatments but obtained the highest IL-10 level, this is consistent with the theory that IL-12 is an intermediary inhibiting IL-12 production. IL-10 is a potent inhibitor of IL-12 production of accessory cells [29].

The results of this invention show that β-Glucan can increase IL-10 level. IL-10 is known for its ability to inhibit activation and effector functions of T cells, monocytes, and macrophages. The main function of IL-10 in the body's immune system is to inhibit the Th-1 response. IL-10 is a type 2 cytokine that stimulates humoral immunity by stimulating the development and activation of mast cells and eosinophils, and differentiating B cells to secrete antibodies and switching immunoglobulin B cells to IgE. IL-10 is a very important cytokine because it inhibits macrophage activation, T cell proliferation, and pro-inflammatory cytokine production.

The cytokines secreted by Th1 are IL-2, IFN-y, and TNF-α that encourage cellular immunity, whereas the cytokines secreted by Th-2 are IL-4, IL-5, IL-6, and IL-10. IFNγ secretion will inhibit Th-2 cells while IL-10 secretion will inhibit Th-1 cells. The results of this study theoretically agree that the presence of protein antigens producing TCD4+ cell responses stimulates differentiation of both Th-1 and Th-2. In the treatment groups, the combination of liposomal β-glucan and liposomal AdhO36 obtained higher average levels than healthy and infected controls. This shows that the potential of β-glucan in increasing IL-10 levels. IL-10 is known for its ability to inhibit the activation and function of T-cell, monocyte, and macrophage effectors. The main function of IL-10 in the body's immune system is to inhibit the Th-1 response.

Research results obtained that in treatment E (β-Glucan + AdhO36) the average IL-10 level was the highest and statistically significant compared to other treatments. The cytokines secreted by Th1 are IL-2 and IFN-y while the cytokines secreted by Th-2 are IL-4, IL-5, IL-6, and IL-10. IFN-γ secretion will inhibit Th-2 cells while IL-10 secretion will inhibit TH-1 cells. The results of this study theoretically agree that the presence of protein antigens producing TCD4+ cell responses stimulates the differentiation of both Th-1 and Th-2. In the liposomal β-glucan and liposomal AdhO36 treatment group, each level was found to

be higher than the healthy or infected controls. This shows that the potential of β-glucan in increasing IL-10 levels. IL-10 is known for its ability to inhibit activation and function of T-cell, monocyte and, macrophage effectors. The main function of IL-10 in the body's immune system is to inhibit the Th-1 response. IL-10 is a type 2 cytokine that stimulates humoral immunity by stimulating the development and activation of mast cells and eosinophils, differentiation of B cells to secrete antibodies and switching immunoglobulin B cells to IgE. IL-10 is a very important cytokine because it inhibits macrophage activation, T cell proliferation, and pro-inflammatory cytokine production [30].

The cytokines secreted by Th2 are IL-4, IL-5, IL-6, and IL-10. From the results of the *in vivo* test, IL-10 obtained by treatment Group E (combination of beta-glucans and AdhO36) increased. IL-10 expressed can come from antigen-presenting cell (APC) which is activated by an antigen, in this case AdhO36. Naive CD+T cell differentiation into Th2 cells is stimulated by IL-4, IL-10. From the research results, the administration of β-Glucan has been shown to increase TCD4 + cells [23].

From the histogram of the number of bacterial colonies and ANOVA analysis results, it was found that overall in the treatment groups there were significant differences between in intestine, liver, and spleen ($p = 0.000 < 0.05$) while in MLN there were no significant differences ($p = 0.153 > 0.05$). The results of the *post hoc* Tukey test on the number of bacterial colonies in the intestine, liver, and spleen results showed that there were significant differences ($p = 0.000 < 0.05$) in the number of colonies with (C) liposomal β-glucan treatment, (D) liposomal AdhO36, and (E) combination of β-glucan and AdhO36 liposomes against infected control (B).

Protection test against bacterial invasion from the research results of (C) liposomal β-glucan, (D) liposomal AdhO36 treatment, and (E) combination of liposomal β-glucan and AdhO36 shows that the treatment can reduce or colonization of bacteria does not occur in the intestine, liver, lien, and MLN. In infected control mice, the amount of colonization in the intestine is very high, while in the liver and in the spleen, the amount of colonization is quite a lot, but in MLN there is little colonization. The results showed no colonization in the intestine, liver, spleen, and MLN. This shows that the treatment can inhibit the invasion of bacteria into organs.

From the results of the study, the administration of liposomal β-glucan treatment provides good protection in inhibiting colonization in the intestine, liver, spleen, and MLN. This is different from the results of the study by Winarsih *et al*. (2019), administration of β-glucan extract from the cell wall of *C. albicans* 300 μg/day 1 time on day 5–day 9 after the mice were infected with *S.* Typhimurium orally 10^8 cells/mL on day 1 and day 3 indicates colonization. Liposomal B-glucan formulation gives better results, this could be due to the particle size in the nanoform that allows cellular uptake and is specific so that the nanoparticle liposomal β-glucan is more effective in uptake for migration by DC. The charge on the liposomal cationic surface can also change the surface of the anionic membrane cell so that this interaction between electro-statistics leads to an internalization efficiency by APC [31]. From biopharmaceutical research, it showed that B-glucan affects the immune system through binding of the cell receptor of APC named Dectin-1 and TLR-2 [18]. This APC will serve antigen to lymphocyte, and the immune system of the body will be inducted to fight infection.

Potential of β-Glucan as immunoadjuvant is proven; the combination of liposomal β-glucan and AdhO36 provides protection and inhibits invasion and colonization in the intestine, liver, spleen, and MLN. B-glucan from several studies provides evidence of immunoadjuvant because it is used to increase the efficacy of vaccines and can stimulate specific immunity. Immunoadjuvant is used in cellular immunity and humoral immunity. The immune response mechanism starts from the APC. This is a crucial stage in activating the immune system. The best APC responsible for activation of T cells, NK cells, and B cells is DC (Jin *et al*., 2018; Patel, 2012). Two main types of cellular immune reactions that eliminate different types of microbes: CD4+ helper T cells + secrete cytokines that recruit and activate other leukocytes to phagocytosis (digest) and destroy microbes, and CD8+ cytotoxic T lymphocytes kill infected cells and contain other microbial proteins in the cytosol, eliminating cellular sources of infection. Cellular immunity protects against infections caused by intracellular bacteria. From the research results, the Th1/Th2 ratio where Th1% Gated (UR) is lower than Th2, 0.582 compared to 1.034, respectively (Figures 3 and 4). Th-1 cells secrete INF-γ, IL-2, and TNF-β which encourage cellular immunity. IL-12 is produced by APCs and activated monocytes or macrophages. IL-12 and TNF-α encourage Th-1 cell responses and cellular immunity. Th-2 cells secrete IL-4, IL-10, and IL-13 which encourage the humoral immune response. Th-1 and Th-2 responses alternately inhibit their secretion [30]. The presence of β-glucan increases the activation of macrophages and DC (Kim *et al*., 2011); this indirectly proves that IL-12 secretion increases with β-Glucan and increases Th-1, an increase in Th-1 will be downregulation by IL-10. Results in Figure 2 show the highest levels of IL-10 in the combination treatment of liposomal β-glucan and AdhO36. Increased IL-10 will increase the humoral immune response, and the colonization test proved to be no colonization in the intestine, liver, spleen, and MLN.

From the results of the study shown in Figure 2, in the treatment (E) combination of liposomal β-glucan and AdhO36, IL-10 level is smaller than the treatment (D). This is likely due to the interaction of physics, chemistry, and pharmacology. This can be seen from

the results of β-Glucan studies on transport in the gastrointestinal tract that shows the transfer rate to its gut ranges between 0.5 and 12 h. The physicochemical characteristics of β-glucan determine this transfer rate. From the results of the flow analysis, β-glucan was added in cells after 24 h. The first cells that can swallow glucan are the small intestinal epithelial cells and only 10% can internalize; this is likely a specific subpopulation where intestinal cells can transfer glucan [32]. From the results of the study above, the characteristics of β-glucan from the *C. albicans* cell wall to determine β-Glucan transport in the small intestine are needed. The results of this study indicate that the potential of β-glucan as immunoadjuvant is proven by increasing cellular immune response and humoral immune response. However, it is needed to develop oral β-glucan liposomal preparations added to AdhO36 related to physical, chemical, and pharmacological interactions to obtain an oral typhoid fever vaccine that is stable, comfortable, safe, and effective and has high efficacy.

Conclusion

The administration of a combination of β-glucan liposome from *C. albicans* and AdO36 *Salmonella* Typhi has a potential effect on cellular and humoral immune response. The β-glucan potential of *C. albicans* as immunoadjuvant has been shown to increase the immune response.

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