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Enhance of IL-22 expression in Oral Candidiasis Immunosupressed Model with *Acanthus ilicifolius* Extract Therapy

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Abstract. Oral candidiasis is an inflammation of the oral mucosa caused by a fungal invasion especially *Candida albicans*, which need the predisposing factors such as Immunosuppression condition. IL-22 plays a role in neutrophil recruitment which plays a role in the defense mechanism against fungal infections. The leaves of *Acanthus ilicifolius* (*A. ilicifolius)* have antifungal potency and antioxidant effects. **Objective.** To know the effect of leaves of *A.ilicifolius*'*s* in chloroform extracts on therapy in enhancing IL-22 expression in oral candidiasis immunosupressed model. **Methods.** This study was true experimental with post test only control group design. Sixteen males of Rattus Novergicus Wistar strain were immunosuppressed with dexamethasone (0.5mg/day) and tetracycline (1mg/day) orally for 21 days, then induced with *C.albicans (ATCC-10231)* $6x10^8$ on the tongue of rats for 2 weeks. Rats divided into four groups (n=4/group): no-treatment (G1), nystatin-treatment (G2), *A. ilicifollius* (8%)-treatment (G3), and *A. ilicifollius* (16%)-treatment (G4). The rats were treated for 14 days, then the tongue were biopsied. IL-22 expression were examined by immunohistochemistry and observed using microscope (400x magnification) and statistically analyzed (One-way ANOVA, LSD-test, $p<0.05$). **Results.** There was significant differences between G1 to G2, G3, G4 ($p<0.05$). There was no significant differences between G2, G3 and G4 ($p>0.05$). **Conclusions.** Chloroform extract from the leaves of *A. ilicifolius* can increase expression of IL-22 in oral candidiasis immunosupressed model. *A. ilicifolius* 8% effective in increasing expression of IL-22 and have same effect in comparison both nystatin and *A. ilicifolius* 16%.

Keywords: immunosupressed, *Candida albicans,* nystatin, antifungal, IL-22

1. Introduction

Candida albicans, known as opportunistic pathogens, is responsible for the vast majority of infections (more than 90%), include oral candidiasis [1]. Several predisposing factors of oral candidiasis include chronic local irritants or mucosal trauma, oral ecology or marked changes in the oral microbial flora (by antibiotics, corticosteroids, immunosuppressives and xerostomia), dietary factors, Immunological and endocrine disorders (e.g. diabetes mellitus, HIV), malignant and chronic diseases, Severe blood dyscrasias, radiation to the head and neck, abnormal nutrition age (e.g. very young or very old), hospitalization, oral epithelial dysplasia, and heavy smoking [1,2]. The expression of *C. albicans* proliferation in oral cavity are strongly correlated with decreasing of the immune system [3,4]. Antonio et al said that *C. albicans* as opportunistic fungus are become a major cause of oral and esophageal infections in immunocompromised patients and affect mostly 90% of patient with human immunodeficiency virus infection or AIDS [5]. Specific depiction of *C. albicans* in oral candidiasis include their ability to adhere and colonize in oral mucosa [3].

Neutrophils have been identified as an important source of both IL-17 and IL-22. IL-22 as a member of IL-10 cytokin family, plays critical role in innate immune defense and mucosal protection from damage. IL-22 has been shown to promote barrier defense and wound healing by secreting antimicrobial proteins, epithelial differentiation, and goblet cell activation in intestinal epithelial cells. Previous study reported IL-22–producing neutrophils were protective during acute coilitis [6]. In defense mechanisms to fungal, IL-22

that can be produced by Th17 cells, was critically involved in the control of *Candida's* growth at mucosal surface, eventhough in defective condition of Th-adaptive immunity. IL-22 can compensites in IL-17 deficiency in mice with mucosal candidiasis [7]. The experiment conducted by Luca et al showed that IL-22 had important contribution in early defense to yeast. IL-22 induce neutrophils recruitment and activate neutrophils, ephitelial cells, and induce releasing of antifungal ß-defensins [8]. These antifungal ß-defensins have potent fungicidal activity that enable the epithelium to prevent invasion of *C.albicans* [9].

A. ilicifolius is one of spiny plant which mostly found in mangrove of Asia and Africa. In traditional practices, these plants have been used to treat rheumatism, asthma, paralysis, psoriasis, and leucorrhoea. It has been reported the antioxidant, hepatoprotective, leishmanicidal, tumour reducing and anticancer activities of various extract of *A. ilicifolius*. The present study showed the antibacterial and antifungal activities of alcoholic, butanolic, and chloroform extracts of leaves and roots of *A. Ilicifolius* [10].Chloroform and n-hexane extracts of *A. ilicifolius's* leaf have strong inhibition againts *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Aspergilus fumigatus*, and *Aspergilus niger* [11]. Previous study showed that chloroform extract of *A. ilicifolius* leaf 1% effective in inhibiting *C. albicans* on thermoplastic nylon soaking [12].The recent studies showed that 8% and 16% *A. ilicifolius* extract can increase the expression of IL-17 and TLR-2 in oral-candidiasis-immunosupressed-mice, which may lead to the improvement of healing process of oral candidiasis [13,14].

Herein, we report the effect of this plant as the basic ingredient of alternative medicine from natural products for oral candidiasis. It is important to be able to compare the effect of *A.ilicifolius* leaf chloroform extracts and nystatin treatment on IL-22 enhance in oral candidiasis immunosupressed model.

2. Experimental Method

2.1. Animal Model

Sixteen healthy of Rattus Novergicus Wistar strain aged 12 weeks, ±250 grams in weight was immunosuppressed by giving dexamethasone 0.5mg/day and tetracycline 1%/day orally for 21 days. The induction of *C. albicans* (ATCC-10231) for candidiasis model by giving $6x10^8$ as much as 0.1 cc then applied on the tounge of rats using sterile cotton bud three times a week for 2 weeks [15].The groups divided into 4 groups (n=4/group), group-1 (G1): induced by *C. albicans* and 0,1% CMCNa (oral candidiasis group), group-2 (G2): induced by *C. albicans* and treated with nystatin topical, group-3 (G3): induced by *C. albicans* and treated with 8% *A. ilicifolius* chloroform extract topically, group-4(G4): induced by *C. albicans* and treated with 16% *A. ilicifolius* chloroform extract topically.

2.2. A. ilicifolius Choroform Extract

Fresh *A. ilicifolius* leaves from Wonorejo Surabaya, then dried in the free air for 2 days.Ten grams of *A. ilicifolius* dried leaves soak for 5 hours with 200 ml chloroform. The solvent was removed and filtered with Whatman's no.1 filter paper then evaporated at low pressure by using Buchi Rotavapor R-200 at 45°C in the refrigerator for further use of the chloroform extract [16].

2.3. Treatment and Data Analyzed

Nystatin as control groups (G2) was applied on tongue surface 0.5 cc at the same hours for 14 days as 8% (G3) and 16% (G4) *A. ilicifolius* chloroform extract. After treating for 14 days, the rats in each group then were euthanized and biopsed. IL-22 expression were examined using immunohistochemical staining method and then observed using light microscope with 400x magnification. We used One-way ANOVA then continue with LSD statistical analysis.

3. Results and Discussion

Determination the effect of chloroform extracts from the leaves of *A.ilicifolius* as therapy in oral candidiasis immunosupressed model.The examination of cytokines that involved in mechanism defences to *C. albicans* and also evaluated the production of cytokine in oral candidiasis immunosuppressed condition without treatment. IL-22 also known has role in the maintenance of mucosal homeostasis and preservation of barrier sites [17].The expression of IL-22 in this study was investigated by examining IL-22 in epitel of

the tongue with immunohistochemical staining (figure 1), and the amount of IL-22 was performed by counting the brown cell on the slide (figure 2).

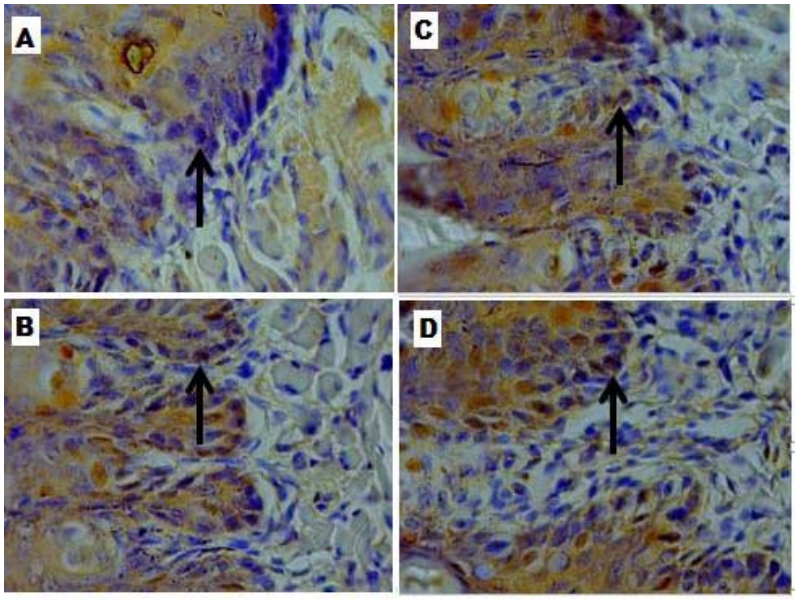

Figure 1. The expression of IL-22 in tongue of Rattus Novergicus Wistar strain (black arrow). A: Oral candidiasis group with no treatment (G1), B: Oral candidiasis group and treated with nystatin topical (G2), C: Oral candidiasis group and treated with 8% *A. ilicifolius* chloroform extract topically (G3), D: Oral candidiasis group and treated with 16% *A. ilicifolius* chloroform extract topically (G4) (Magnification 400x).

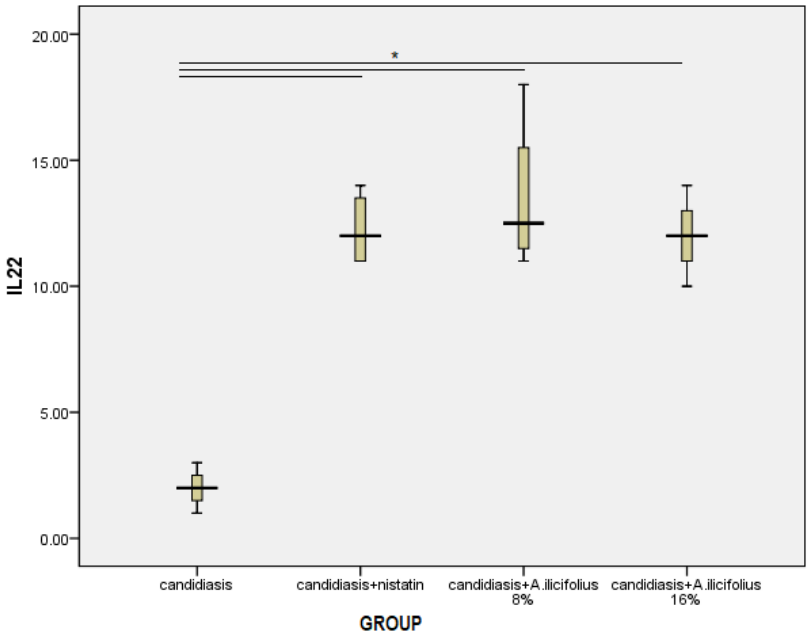

Figure 2.IL-22 were expressed as mean ± Standart Deviasion in each group (*p<0.05).

Figure 2 showed that the lowest expression of IL-22 was on the G1 (2.00 ± 0.41) compared to G2 (12.25 ± 0.75), G3(13.5 ± 1.55) and G4(12.00 ± 0.82), while the highest expression of TLR-2 showed in group of rats which were induced by *C. albicans* and treated with *A. ilicifolius* chloroform extract 8% topically (G4) compared to other groups. The data from G1 on IL-22 expression in this study indicated that on immunosuppressed condition with oral candidiasis, the low amount of IL-22 may lead to aggravate fungal infection. Eyerich et al reported that patients with Cronic Mucocutaneus Candidiasis produced significantly lower amounts of IL-17 and IL-22 mRNA compare with healthy patients [18].The important role of IL-22 has been reported as a first-line defense in candidiasis by critically controlling initial fungal growth and epithelial homeostasis in the relative absence of Th1 immunity, and also produced by innate and adaptive immune cells in candidiasis [8]. Dexamethasone it self blocks naïve Tcell proliferation and differentiation where used as immunotherapy [18], and in this study affected to IL-22 production. The expression of IL-22 from other groups with treatment was enhance due to the treatment.

The topical use of nystatin is considered the most commonly used for oral candidiasis treatment in dentistry and also plays an important role in the prophylaxis of oral and systemic candidiasis in full-term and premature newborns, infants, and immunocompromised patients [20].Nystatin is an antifungal compound with potent proinflammatory properties and reported induces interleukin (IL)-1β, IL-8, and tumor necrosis factor alpha secretion via TLR-1 and TLR-2 [21]. In this study, expression of IL-22 enhance significantly compare to oral candidiasis with no treatment ($p<0.05$). Although it has an important role as antifungal and be able induces proinflamatory cytokines, it reported to be resistant to oral candidiasis [22]. Therefore, innovation to make anti-fungal drug from natural product seems promising.

Expression of IL-22 in treatment groups with 8% and 16% *A. ilicifolius* chloroform extract in this study enhance significantly compare to oral candidiasis with no treatment ($p<0.05$), while there was no significant differences between nystatin treatment ($p>0.05$).No significant difference between treatment groups with 8% and 16% *A. ilicifolius* chloroform extract ($p>0.05$) also reported in this study. This indicate that the treatment of 8% *A. ilicifolius* chloroform extract for 2 weeks was able to enhance IL-22 and has same effect with nystatin. The recent study, this extract could increased the expression of IL-17, that may play a role in the pathway of antifungal immune system. The cytokines IL-22, the member of IL10 and which is also produced by Th17 cells,reported has a more crucial role than IL-17 in mucosal host defense to *C. albicans* by controlling the growth of yeasts as well as by contributing to the host's epithelial integrity in the absence of acquired Th1-type immunity [18,23-25].The impaired or absence of T helper-1 (Th1) immune response leads to increased susceptibility fungal infections severity [26].

The anti-microbial activity of chloroform *A. ilicifolius* leaves extracts were studied and exhibited strong inhibitory action against *Candida albicans* [27]. Pentacyclic triterpenoids and sterols were isolated and characterized from *A.ilicifolius* chloroform extract [11]. Pentacyclic triterpenoids revealed as antimicrobial activity, while sterol as antioxidant. Recent study reported that *A. ilicifolius* chloroform extract has total phenolic compounds that known to be good natural anti-oxidants with total phenol (70.833±1.071mg/g), total flavonoids (50.733±0.851mg/g) and total tannins (35.367±0.328mg/g) [28].That bioactive components are utilization as potential natural antimicrobial and antioxidant.

4. Conclusion

The expressions of IL-22 are higher after treated with *A.ilicifolius* chloroform extract and nystatin, compared by no treatment. The results showed that 8% and 16% *A.ilicifolius* chloroform extract were effective in increasing expression of IL-22 in oral Candidiasis immunosupressed model. *A.ilicifolius* chloroform extract has antifungal effect and have similiar effect compare with nystatin. 8% *A. ilicifolius* chloroform extract is the best concentration in increasing expression of IL-22.

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