Effectiveness of Giving Secang Wood Extract (Caesalpinia Sappan L) Against IL-6 And IL-10 Levels in Balb / C Mice With Vulvovaginalis Candidiasis

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Abstract

The aim of this study was to test the effectiveness of secang wood extract *(Caesalpinia* sappan L) on IL-6 and IL-10 expression in Balb / c mice with vulvovaginalis candidiasis. This type of research is an experimental laboratory, with a control group design using experimental animals as research objects. The research subjects were BALB / c mice weighing 35-40 grams, aged 10-12 weeks, the number of experimental animals needed in this study was 18 for 3 random groups, each group being 6. Analysis and data processing using the excel program SPSS Repeated ANOVA Test was used to measure differences in cytokine levels. IL-6 levels in group 1 (control) where the value of IL-6 expression after infection (H1) was 4309.40 pg /mL (IQR value 3803.8-4963.5) after treatment (H7) in the control group increased by 5578.12 pq / mL (IQR 5330.3 - 6162.9). In group 2 that were given fluconazole and secang wood extract, IL-6 levels tended to decrease by 3516.4 pq / mL (IQR: 3070.4-4061.6) Secang wood after infection decreased from 3417.34 pq / mL to 2782.97 pq / mL after treatment. In Group 2 that was given fluconazole and secang wood extract, IL-10 levels increased by 1414.8 pq / ml (IQR: 1061.7- 3158) after infection, to 3268.35 pq / ml (IQR value: 1789.9-3709.6) after treatment. given secang wood after infection increased from 2032.67 pq / ml to 3091.83 pq / mL after treatment. The p value is 0.008. Obtained the effect of secang wood extract in reducing levels of Interleukin 6 and can increase the value of IL-10 levels after administration of secang wood extract on candidiasis vulvovaginalis

Keywords: *Secang wood, IL-6, IL-10, vulvovaginalis candidiasis*

Introduction

According the Research shows that 75% of women have had one episode of vulvovaginal candidiasis (CVV) and another 40-45% have two or more episodes of vulvovaginal candidiasis (KVV) in their lifetime,[1,2.] Vulvovaginal candidiasis (KKV) is commonly known as vaginal discharge. by the community and is a quite disturbing problem. Chronic vulvovaginal candidiasis is also known to be a triggering factor for vulvovaginalis.[3,4] The World Health Organization (WHO) is a problem that should be regularly investigated because it reduces the quality of life for women and their partners[5,6].

Common symptoms are pain in the vaginal area, irritation, burning sensation, dyspareunia, and pain when urinating, which begins with acute pruritus and vaginal discharge (fluor albus). Clinical manifestations of candidiasis vulvovaginalis are the interactions between the pathogenicity of Candida species and the host defense mechanism which is related and influenced by several predisposing factors. Treatment

of conventional KKV vulvovaginal candidiasis (candidiasis vulvovaginalis) with systemic and topical drugs. Generally, Systemic therapy uses a single dose of 1x150mg flukoazole. While topical uses Ketocenazol by rubbing it on the lesion[7,8,9.]

Interleukin 6 (IL-6) is mostly considered a pro-inflammatory cytokine, but it also has regenerative and anti-inflammatory activity. While IL-10 is a cytokine that has the main function of limiting and terminating the immune response (anti-inflammatory). IL-10 cytokines, which are anti-inflammatory cytokines, during this candida infection condition it can increase phagocytosis and neutrophil recruitment so as to mediate inflammation [1,2,3] . Cytokines inhibit the activity of Th2 cells, NK cells and magrophages. When pathogens are able to withstand the destruction of normal immune mechanisms. If an IL-10 infection occurs, it will be produced to reduce inflammation which will minimize pathological conditions due to excessive inflammation[4,5,6].

The use of herbal ingredients as alternative medicines in healing diseases is increasing. This is because the therapeutic effects of herbal ingredients are constructive, the side effects that are caused are very small so that herbal ingredients are relatively safer from chemicals[3,8,9.] Currently, there are known treatments with Chinese herbal medicines which include Syngonanthusnitens, Euphorbia hirta L, Centellaasiatica, Cymbopogoncitratus (DC) Stapf (Gramineae), Areca Cathechu, L. Piper Betle L., Terminaliacatappa shows a deep[3,10,11.]

Secang wood has an anti-fungal effect against the fungus Candida Albicans allegedly due to the active substances in secang wood that are soluble in ethanol[5,9,12] The main active substances contained in secang wood include polyphenolic compounds, namely tannins and brazilins The extract of secang wood (Caesalpinia sappan) showed the presence of tannins and alkaloids. Among gram-positive and gram-negative bacteria, gram-positive strains of bacteria were more susceptible to extracts when compared to gram-negative bacteria. This may be due to the fact that these two groups differ in the structure of their cell wall components. The ability of tannin compounds to cause bacterial colonies to disintegrate, most likely due to their interference with the bacterial cell wall; thus inhibiting microbial growth .

Materal and Methods

The type of research used is pure experimental, namely laboratory experiments, pretest - posttest control group design using experimental animals as research objects. The research subjects were BALB / c mice weighing 35-40 grams, aged 10-12 weeks. healthy and fulfilling the inclusion criteria. The sample size was divided into 3 groups randomly in each group at least 5 (n = 5) and added by 1 animal for each group as a reserve so that the number of experimental animals needed in this study was 18 animals for 3 random groups, each group being 6.

a. Group (negative control) the group given distilled water and not given secang wood extract and intravaginal stimulation with candida albicans

b. Group 2 (positive control): the group that was given anti-candida drug (Fluconazol) at a dose of 19.5 mg / kgb was stimulated by intravaginal candida albicans

c. Group 3 (intervention): Given the extract of secang wood (Caesalpinia sappan L) at a dose of 510 mg / kg body weight and stimulated with candida albicans intravagina.

The Analysis and data processing using The excel program SPSS Repeated ANOVA Test was used to measure differences in cytokine levels in repeated measurements of each group. The results of the study were considered significant if the p value was <0.05. Research data will be presented in tables and graphs

Result

Table 1 : IL-6 Levels After Being Infected (H1) Given Candida Albicans And After 7 Days Of Treatment

Group	Post Infection (H1) Median (IQR) (Fc)	Post Treatment (H7) Median (IQR (Fc)	Mean	p
Group 1 (Negatif Control)	4309.40 (3803,8 – 4963.5)	5578.12 (5330.3 –6162.9)	1268.72	0.003
Group 2 (Positive Control) Flukonazole 19.5 mg/kgBB	3516.46 (3070.4 – 4061.6)	1890.91 (1464.6 – 2346.8)	-1942.73	
Group 3 (Intervensi) Secang wood	3417.34 (3040.6 – 3952.5)	2782.97 (2584.7 – 3030.7)	-317.18	

According table 1 The results showed that group 1 (control) post infection (H1) obtained IL-6 levels: 4309.40 pg / mL (IQR values 3803.8 - 4963.5) post treatment (H7) IL-6 levels in the control group increased by 5578.12 pg / mL (IQR values 5330.3 - 6162.9) with an average value (mean) of 1268.72 Furthermore, in the group given secang wood and fluconazole a dose of 19.5 mg / Kgbb post infection, the IL-6 level value of 3516.46 pg / mL (IQR 3070.4 – 4061.6) decreased to 1890.91 pg / mL (IQR 1464.6-2346.8) post treatment. Furthermore, group 3 obtained the results of IL-6 levels of 3417.3 pg / mL (IQR 3040.6 – 3952.5) decreased to 2782.9 pg / mL (IQR 2584.7 – 3030.7)

Table 2: IL-10 levels, after being infected (H1) given candida albicans and after 7 days of treatment

Group	Post Infection (H1) Median (IQR) (Fc)	Post Treatment (H7) Median (IQR (Fc)	Mean	p
Group 1 (Negatif Control)	4018.60 (3268.3 – 5828.0)	2871.17 (1988.5 – 3775.8)	-1368.09	0.008
Group 2 (Positif Control) Flukonazole 19.5 mg/ kgBB	1414.82 (1061.7 – 3158.0)	3268.35 (1789.9 – 3709.6)	529.58	
Group 3 (Intervensi) Secang wood	2032.67 (819.03 – 2694.6)	3091.83 (2429.8 – 3709.6)	1279.83	

According table 2 The result showed that group 1 (control) post infection (H1) obtained IL-10 levels of 4018.60 pq / ml (IQR values 3268.3 - 5828.0) post treatment (H7) IL-10 levels in the control group decreased by 2871.17 pq / ml (IQR values 1988.5 - 3775.8) with an average value (mean) of -1368.09. Furthermore, in group 2 who were given secang wood and fluconazole at a dose of 19.5 mg / KgBW after infection (H1) the IL-10 level of 1414.82 pq / ml (IQR 1061.7-3158.0) increased to 3268.35 pq / ml (IQR (1789.9 - 3709.6) after treatment (H7) .

Discussion

The high content of flavonoids in secang wood extract (Caesalpinia sappan L.) of 6.02% influenced the strong anti-fungal activity. Secang wood extract (Caesalpinia sappan L.) also contains 2.43% anthocyanins. Apart from being good antioxidants, anthocyanins can also act as antimicrobials. The factor which also influenced the increase in inhibition diameter was due to the presence of the concentration of antimicrobial substances which increased with each concentration. In addition, the ability of the antifungal activity of secang wood extract (Caesalpinia sappan L.) is due to the fact that secang wood positively contains other secondary metabolites compounds that also act as antifungal activity. According research the ethanol extract of secang wood (Caesalpinia sappan L.) is positive for flavonoids, saponins, alkaloids, tannins, phenolics, triterpenoids, steroids and glycosides. These metabolite compounds are able to act as good antifungals[5,7,13,14].

The mechanism of action of flavonoids as antifungal compounds is divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism In inhibiting the synthesis of nucleic acids, the A and B rings of flavonoid compounds play an important role in the intercellation process or hydrogen bonding, namely by accumulating nucleic acid bases so that they inhibit the formation of DNA and RNA[3,7,915]. The results of flavonoid interactions will also cause damage to cell wall permeability. In inhibiting the function of the cell membrane flavonoids will form complex compounds from extracellular and dissolved proteins so that the cell membrane will be damaged and intracellular compounds will come out. Meanwhile, in inhibiting energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing the formation of energy in the cytoplasmic membrane and inhibiting the motility of bacteria that play a role in antimicrobial activity and extracellular proteins[2,16..]

Inflammation is a response to injury, where there is accumulation of leukocytes, inflammatory mediators such as cytokines. Inflamation occurs in acute and subacute / chronic stages. In the inflammatory stage, proinflammatory cytokines including IL-6 are released. In the early stages, the infection takes the form of Candida. Planktonic albicans occurs by introduction of yeast immunity to mice. Introduction of the immune system through PAMPs molecules derived from, which bind C. albicans to the receptors (PRRs) on the surface of polymorphonuclear (PMN) cells in the intestinal mucosa[4,8,10.]

The cell surface contains β-1,3- Candida glucan sugar groups which act as PAMPs (17). Levels of Cytokine nterleukin-6 (IL-6) Serum I The process of introducing PAMPs and PRRS, namely in the early phase of infection, occurred on days 7 and 14 of candida abicans after

inoculation which was marked by no C. albicans discovery of secretion of the cytokine IL-6 (0pg / ml). The next step occurs when the bond between the sugar group and the receptor on PMN cells initiates the release of cytokines including IL-6[.5,8,17]

This study is similar to the study conducted by Masfufatun's, putu which states that inflammation is a response to injury, where there is an accumulation of leukocytes, inflammatory mediators such as cytokines. Inflammation occurs in acute and subacute / chronic stages. In the inflammatory stage, proinflammatory cytokines including IL-6 are released. In the early stages, the infection takes the form of Candida. Planktonic albicans occurs by introduction of yeast immunity to mice. Levels of Cytokine nterleukin-6 (IL-6) Serum I The process of introducing PAMPs and PRRS, namely in the early phase of infection, occurred on the 7th and 14th day of candida abicans after inoculation which was marked by no C. albicans discovery of secretion of the cytokine IL-6 (0pg / ml). The next step occurs when the bond between the sugar group and the receptor on PMN cells initiates the release of cytokines including IL-6.

In this study, the value of IL-6 levels increased in group 2 which was given fluconazole and secang wood extract. and group 3 who were given only secang wood. According to Couper KN states that serum levels of anti-inflammatory cytokines (IL-10) In infectious conditions, very candida pro-inflammatory cytokines play a role in increasing phagocytosis, neutrophil recruitment, thus mediating inflammation.[18,21,22,23] The IL-10 cytokine is an anti-inflammatory cytokine. During infection, these cytokines will inhibit the activity of Th2 cells, NK cells and marophages. When the pathogen is still able to withstand destruction through normal

immune mechanisms, IL-10 will be produced to reduce inflammation which in turn minimizes pathological conditions due to excessive inflammation.[11,24,25]

Conclution

obtained the effect of secang wood extract can reduce levels of interleukin IL-6. The addition of secang wood extract with fluconazole provided a better reduction effect which could be observed in this study. It was also found that there was an effect of secang wood extract on increasing IL-10 levels.

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Conflict of Interest- None of the authors hascompeting interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University.

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