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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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bstract:

Objective: 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.

Method .: 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

Result: IL-6 levels in the control group where the value during the treatment period (10 days) was 4368,874 to 572,922 after being given the secang wood extract. Compared to the group that was given fluconazole, the IL-6 level tended to decrease by 3556,104 post-treatment to 1902.804 p value> 0.002. whereas the group that was only given secang wood decreased SD 472,746 to 247,596. The value of IL-10 levels during the treatment period of 1970,880 (SD: 1189,762) became 2853,520 (SD = 1001,318) after 7 days of treatment. And the p value> 0.94. Whereas in the group given the secang wood treatment during the treatment period, the IL-10 level value of 1812.005 became 3074,180 after administration. p value is(> 0.11).

Conclution :. Obtained the effect of secang wood extract in reducing IL-6 levels. The addition of secang wood extract with fluconazole did not give a better reduction effect. whereas at IL-10 levels, there was no effect of secang wood extract.

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

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[7,8,9,10].

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[7,8,9.] 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 role[3,10,11,12.]

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. 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 .

MATERIALS 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 1 (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.
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 (negative control) the group given distilled water and not given secang wood extract and intravaginal stimulation with candida albicans

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 relationship between variables was analyzed using the Pearson correlation test. 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

This study used 25 mice albino selected randomly and were divided into 5 treatment group consisting of 5 group : UVB alone, UVB with base cream, UVB with mangosten pericarp extract cream wit concentration of 1 %, 3 % and 5 %. From the result of the ELISA levels of 8-OHdG was found that there are significant difference as between the various treatment groups (p>0,05) as evidence by ANOVA (Table 1).However, the increasing concentration of mangosten pericarp extract cream showed decreased level of 8-OHdG. The level of 8-OHdG highest in UVb control group and lowest in mangosten pericarp extract cream 5 % groups.

Table 1 IL-6 levels after infection (H0) after being given Candida albicans and after treatment (H8)

	Pre treatment [10 days]	Post Treatment [7 days)	Different on average	p- value
Control	4368,874 (SD = 605,029)	5712.922 (SD = 428,714)	1344,048	0,026
Flukonazol	3556,104 (SD = 536,526)	1902,804 (SD = 472,993)	-1653,300	0,002
Kayu secang	3480,774 (SD = 472,746)	2802,804 (SD = 247,596)	-677,970	0,059

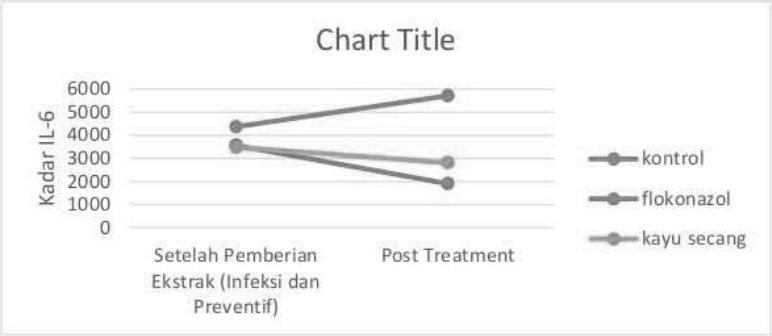
According table 1 During the treatment period the IL-6 level tended to increase during the treatment period compared to after completion of treatment. This can be seen in the control group where the value during the treatment period (10 days) was 4368,874 to 572,922 after being given secang wood extract. Compared to the group that was given fluconazole, the IL-6 level tended to decrease by 3556,104 post-treatment to 1902.804 p value> 0.002. while the group that was only given secang wood decreased by SD 472,746 to 247,596

Table 2 IL-10 Levels After Infection (H0) After Being Given *Candida Albicans* And After Treatment (H8)

	Pre treatment [10 days)	Post treatment [7 days]	Different on average	p– value
Control	4442,270 (SD = 1328,585)	2879,998 (SD = 913,758)	-1562,272	0,72
Flukonazol	1970,880 (SD = 1189,762)	2853,520 (SD = 1001,318)	882,639	0,94
wood secang	1812,005 (SD = 991,774)	3074,180 (SD = 647,551)	1262,174	0,11

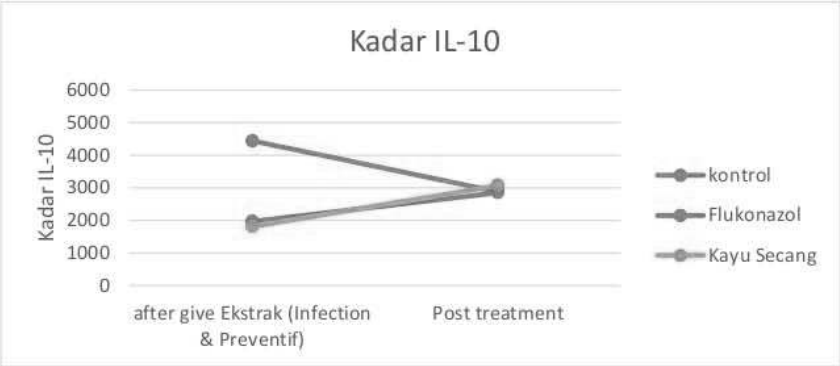
According table 2 In the treatment group for 10 days and given candida infection, the IL-10 levels tended to increase after administration of fluconazole and secang wood treatment. Where the values obtained during the treatment period of 1970,880 (SD: 1189,762) became 2853,520 (SD = 1001,318) after treatment for 7 days was given. And the p value> 0.94. Whereas in the group given the secang wood treatment during the treatment period, the IL-10 level value of 1812.005 became 3074,180 after administration. The mean value is 1262.174 and the p value is> 0.11.

administration. The mean value is 1262.174 and the p value is> 0.11.



Graph 1 Profile of Interleukin 6 Levels during the study

From graph 1 it can be seen that the IL-6 level profile in this data has increased after being given infection and giving secang wood extract. And these results can be seen in the negative control group, while the group that received treatment, both those given fluconazole and secang wood extract and the group that was given only secang wood experienced a decrease. This found a significant difference with p <0.001.



Graph 2 Profile of Interleukin 10 Levels during the study

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 antifungals5,7,13,14.

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[3,7,9,15]. 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 Kovacs, namely that IL-6 levels will increase sharply at 72 hours after infection compared to 24 hours after infection in systemically infected mice. From further research, it was found that the profile of interleukin 10 levels in this data decreased after infection in the control group. In contrast to the group given treatment after administration of the extract and infection, IL-10 levels increased[18,19,20] And the value obtained at the level of interleukin 10 p <0.011 or there is no relationship Serum levels of anti-inflammatory cytokines (IL-10) In conditions of infection, proinflammatory cytokines are very candida that 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 effect of secang wood extract in reducing interleukin IL-6 levels. The addition of secang wood extract with fluconazole was not a better reduction effect that could be observed in this study. It was found that there was no effect of secang wood extract in increasing IL-10 levels. The addition of secang wood extract with the anti-fungal drug fluconazole levofloxacin provided a better enhancing effect which was observed in this study.

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Conflict Of Interest- None of the authors has competing interests

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

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