The Effectiveness of Topical Propolis Administration in the Treatment of Vulvovaginal Candidiasis in Mouse Model Experiments

Ginting, DSM 1, L. Intang, LS 2, Rusda, M 2, Marpaung, J 2, Adenin, I 2, Rivany R 2

1Faculty of Medicine, University of North Sumatra, Medan, Indonesia,
2Department of Obstetry and Gynecology, Faculty of Medicine, University of North Sumatra, Medan Indonesia.

Abstract
Objective: This study is an analytical study with an experimental design, namely the Post Test Only Control Group Design, which assesses the effectiveness of administering Propolis to mice infected with vulvovaginal candidiasis.

Methods: This research was conducted at the Animal House of USU’s FK and Microbiology Laboratory at USU Hospital. This research was conducted from 27 November to 7 December 2019. The study population was 36 experimental mice infected with vulvovaginal candidiasis. The Colony Forming Unit (CFU) was examined on 36 rats divided into 2 groups, namely control and treatment and then examined by CFU every day from Day 0 to Day 4. The data obtained was assessed by the Mann-Whitney hypothesis test.

Results: Data 36 samples were divided into 2 groups found in group A, which did not receive Propolis, the number of CFU from day zero until the fourth day was 92.22 CFU, 128.89 CFU, 78.67 CFU, 56.61 CFU, and 51.61 CFU. Whereas in group B that received Propolis, there was a mean Colony Forming Unit (CFU) that declined from zero to fourth day, namely 97.56 CFU, 15.06 CFU, 2.61 CFU, 0.5 CFU, and on last day obtained 0 CFU. Topical propolis is effective in treating Vulvovaginal Candidiasis (KVV) in experimental model mice where the administration of Propolis can reduce the amount of CFU in the rat's vagina from day to day.

Conclusion: Topical propolis is effective to reduce the number of CFUs in rats infected with vulvovaginal candidiasis (KVV).

Keywords: Propolis, Topical, Vulvovaginal Candidiasis (KVV), Colony Forming Unit (CFU).

Introduction
Vulvovaginal candidiasis (KVV) is an infection caused by Candida species especially Candida albicans although there are other non-Candida albicans Candida (NCAC) species such as Candida glabrata. Although KVV is the second most common vaginal infection, it is not included in sexually transmitted diseases and because candida is considered part of the normal vaginal flora. However, research has confirmed the transmission of candida organisms by vaginal intercourse and other forms of sexual activity. Candida albicans species are part of the normal flora of the vagina but there are many factors that can cause excessive growth leading to candidiasis. How Candida species can cause infection is mediated by a number of virulence factors which include adhesion, biofilm formation, extracellular hydrolytic enzyme production, hyphal formation and phenotypic transition. The diagnosis of KVK requires pelvic examination. The combination of thick white vaginal discharge and vulvar pruritis is not sensitive and is not specific itself for diagnosis. Erythema and edema of the vulva and vaginal tissue, together with thick, white vaginal discharge, support the diagnosis. KVV vaginal secretions have a pH <4.5, and budding yeast and pseudohyphae can be seen on a wet mount. Negative Whiff tests and gram staining can reveal polymorphonuclear cells (PMN) and pseudohyphae. When there is complicated CVV evidence, collecting vaginal fluid for yeast culture and speciation can help with direct therapy. In this study, Colony Forming Unit (CFU) examination was used as a parameter for the success of CVV treatment with topical Propolis. For the quantification of fungal loads, a series of 10-fold serial dilutions was carried out using sterile PBS and plated to the CHROMagar™ plate. CFU is calculated after incubation at 35 °C for 24 hours and expressed as CFU / 100 μl lavage fluid or also called CFU / plate or CFU / plate agar. The most prominent and general aspect that can be relied on with mouse models is that these rodent models and human infections are very dependent on estrogen. Estrogen uses the multifunctional permissive role for vaginal candidiasis through a number of effects both host and Candida. Mice must be placed in a stable pseudoestrous condition to establish an experimental vaginal infection. In mice this is usually achieved by ovariectomy followed by estrogen treatment although recent research shows that a special estrogen regimen is sufficient to allow Candida albicans infection. Of note, ovariectomy has been shown to cause a decrease in the number of lactobacillus components of the vaginal microbiota. In mice, administration of estrogen is a standard treatment by considering the variable susceptibility of different animal strains to estrogen. In both rodents, pseudoestrous induces changes that are highly dependent on the stage in the vaginal epithelium, with a surface of keratinization where the fungal cells are attached and multiply with hyphal growth and eventually form biofilms. In addition to these structural modification properties, other estrogen-induced biological activities appear to have special relevance for CVC / CVC. Specifically, estradiol has been reported to inhibit the differentiation of Th17 cells, a
subset of CD4 cells that should be involved in the mucosal antifungal defense, with strong evidence, however, in the mouth and intestine rather than vaginal infections. Overall, there is evidence that the response to estrogen has similarities in rodents and humans. The basic protocol using mice for KVV research was established in 2014 by the NIH (National Institute of Health), namely:

1. Female CBA / J mice were injected subcutaneously in the lower abdomen (UNIT 1.6) with 0.1-0.5 estradiol valerate in 20-100 μl sesame oil 3-6 days before vaginal inoculation and repeated every week until the study was completed.

2. Prepare a solution of 2.5x10⁶ C. albicans blastospores cells for culture in 0.02 ml of sterile PBS (phosphate-buffered saline).

3. Inoculate a 0.02 solution of fungal cells or sterile PBS (for negative control mice) in the vagina using a micro pipette and a sterile tip. Insert the tip until it runs out into the vaginal lumen, near the cervix. Do it under anesthesia or quickly to minimize stress mice. Anesthetics use ketamine (100 mg / kg rat) and xylazine (10 mg / kg rat) intraperitoneal (UNIT 1.6) in each rat several minutes before inoculation.

4. Perform this vaginal smear on anesthetized mouse (serial measurement on days 4, 7 and 10 after inoculation) and on the last calculation of the rat in euthanasia. The trick, wash the vagina by spraying 100 μL PBS forwards and backs several times. Avoid trauma to the lumen wall when taking serial measurements.

5. Prepare homogeneous serial solution 1:10 there is a sterile PBS. Prepare 3 plates of YPD (Yeast-Extract Peptone Dextrose) with each solution of 25-50 μL. Incubation for 24-48 hours. Count the number of colonies as CFU.

KVV can be induced in mice. Preliminary observations show that the optimal time for induction of vaginal infection is during the estrous stage in mice. The estrous stage is characterized by the appearance of epithelial cells in the vaginal exudate. The estrous-cycle stage of mice is 3-4 days in duration. Mice that are infected during the estrous stage will develop the infection, which can disappear spontaneously afterwards. Therefore, most researchers used a mouse model in a constant state of estrus by inoculating female mice with estradiol benzoate 3-4 days before inoculation with C. albicans. The infection is induced by intravaginal inoculation of 10⁷ C. albicans yeast cells and can be maintained by repeated weekly inoculation of estradiol benzoate.

Candida inoculation in rat vagina. 26

A) Rats are confined for inoculation. Mice are placed on wire inserts and held by the base of the tail, slightly upward to lift the legs and open the vaginal opening. The rat's hips can be stabilized with the same hand when trying to hold the tail restraint.

B) Exposure of the inoculum into the vaginal lumen. The tip of the pipette is gently inserted about 5 mm into the vaginal lumen. The suspension inoculum is then stored.

Propolis is a mixture of complex compounds also called bee glue is a natural resin product collected by bees from several plants and mixed with beeswax and bee saliva that contains enzymes (gl - glucosidase). Bees use propolis in their hives as a protection against predators and microorganisms, to repair damage, as a thermal insulator, and as an aseptic defense to prevent microbial infections in larvae. Propolis is a lipophilic material that is hard and easily breaks when cold but soft, flexible, and very sticky when warm, has an aromatic odor and different colors, including brown, green, and red. In terms of chemical composition consists of 50% resin, 30% wax, 10% essential oil, 5% pollen, and 5% other substances which include minerals and organic compounds such as phenolic acids (cinnamic and caffeic acids) or estern flavonoids (flavones, flavanons, flavonols, and dihydroflavonols chalcones), terpenes, aromatic aldehydes and alcohols, fatty acids, stilbenes, and β- steroids. Propolis in vivo suppresses the lipoxygenase pathway from arachidonic acid metabolism during inflammation. Anti-inflammatory activity of propolis is by inhibiting PGE2 and reducing cytokines. Other mechanisms of propolis that are reported are affecting the activity of inflammatory cells (cell migration, macrophage activation), inhibiting tumor necrosis factor (TNF), reducing nitric oxide synthesis and reducing enzymatic activity during the healing process. From in vitro studies using topical propolis extract it was concluded that both propolis-based gels (G2 and G3) and cream (E3) were partially able to control KVV in mouse models. These results indicate that propolis is a promising and potential alternative therapy for controlling CVC. Considering the fact that propolis is a complex substance with several possible antifungal active compounds, it may be more difficult for resistance to evolve in C. albicans, because it will require several concurrent mutations. In addition, it is possible to increase the therapeutic value by combining propolis with other antifungals such as azoles or caspofungin agents.
promising aspect of this combination therapy is the fact that propolis inhibits the yeast transition to hyphae which contributes to the overall virulence of C. albicans and may even be a target for the development of antifungal drugs.

Method

This study used an experimental design namely the Post Test Only Control Group Design in November-December 2019 by using 36 experimental rats infected with vulvovaginal candidiasis that had been bred in the Department of Mathematics and Natural Sciences Unit of the Animal Lab Unit of North Sumatra University (USU) and CFU examination at the Department of Microbiology Hospital North Sumater University. Then 36 mice were divided into two groups: group 1 was a control group that was not given treatment and group 2 is a group that was given per lakuanc be Propolis topical. The results of this study are presented in the frequency distribution table. To assess the effectiveness of topical propolis administration in mice with vulvovaginal candidiasis, inferential statistical analysis was performed using independent T-Tests. Test if the data is normally distributed. If the data are not normally distributed, the Mann Withney hypothesis test will be used with a confidence level of 95%.

Results

This study analyzed data from the 36 mice that had been infected with Jamus Candida albicans de ngan Propolis Topical administration that includes the inclusion and exclusion criteria, Table 1 gain characteristics of the study subjects a total of 36 samples were obtained 25 mice (59.4%) with a pH of 7.0 and 11 mice (30.6%) with a pH of 8.0. Whereas for body weight, 23 rats (63.9%) with body weight of 90-150 grams and 13 rats (36.1) with body weight of 150-250 grams.

Table 1 Characteristics of research subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>25</td>
<td>69.4</td>
</tr>
<tr>
<td>8.0</td>
<td>11</td>
<td>30.6</td>
</tr>
<tr>
<td>Body weight (grams)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 - 150</td>
<td>23</td>
<td>63.9</td>
</tr>
<tr>
<td>150-250</td>
<td>13</td>
<td>36.1</td>
</tr>
</tbody>
</table>

Table 2 shows that group A, who did not get Propolis, obtained the number of CFUs from zero to fourth day was 92.22 CFU, 128.89 CFU, 78.67 CFU, 56.61 CFU, and 51.61 CFU. Whereas in the group that received Propolis, there was a mean Colony Forming Unit (CFU) which declined from the first day to the fifth day. On zero day, the average CFU of 18 samples was 97.56 CFU, on the first day 128.89 CFU, on the second day 78.67 CFU, on the third day 56.61 CFU, and on the fifth day 0 CFU were obtained. Tables 3 show that the tests conducted with control comparisons are used to assess the effectiveness of antifungal power statistically. On day zero the value of p = 0.499 was obtained, it was concluded that it had no meaning while on day 1,2,3,4 the value of p <0.001 was obtained.

Table 3. Differences Effectiveness Granting Propolis Topical in infected mice KVV Based on Value p

<table>
<thead>
<tr>
<th>Day</th>
<th>Average CFU (Mean)</th>
<th>Value of p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Propolis</td>
</tr>
<tr>
<td>Day 0</td>
<td>92.22</td>
<td>97.56</td>
</tr>
<tr>
<td>Day-1</td>
<td>128.89</td>
<td>15.06</td>
</tr>
<tr>
<td>Day 2</td>
<td>78.67</td>
<td>2.61</td>
</tr>
<tr>
<td>3rd day</td>
<td>56.61</td>
<td>0.5</td>
</tr>
<tr>
<td>4th day</td>
<td>51.61</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mann-Whitney Test (p <0.05)
Discussion

From the research, 36 subjects were obtained. Based on the characteristics of vaginal pH, the majority of study subjects had a vaginal pH of 7.0, as many as 25 subjects (69.4%). Based on research conducted by Vaccia (2017), acidic pH can interfere with Candida growth. Because, at low pH, structural changes occur to the cell wall that makes the host immune system more easily recognize Candida. As a result, the body's immune reaction occurs in acidic conditions, while in alkaline conditions, Candida relatively grows better without an immune reaction from the body.

In this study, it was found that the mean CFU was different between the two groups, that is between group A who did not get Propolis tended to increase CFU and group B who got Propolis decreased CFU starting the first day of Propolis administration. Meanwhile, groups that did not get Propolis tended to experience an increase in CFU. In a study conducted by Capoci (2015), it was found that in a comparative study between two groups, namely the group receiving Propolis and the group not receiving Propolis extract (control), there was a decrease in CFU in all isolate groups receiving Propolis extract. This is because Propolis has the ability to inhibit biofilms which is one of the strong factors of attachment between Candida albicans and vaginal mucosa.

In a study conducted by Wagh (2013) shows that propolis in vivo suppresses the lipoxygenase pathway from arachidonic acid metabolism during inflammation. Anti-inflammatory activity of propolis is by inhibiting PGE2 and reducing cytokines, other mechanisms by influencing inflammatory cells (cell migration, macrophage activation), inhibiting TNF, reducing nitric oxide synthesis and reducing enzymatic activity during the healing process.

The results of McLennan SV (2007) show that propolis inhibits neutrophil and macrophage infiltration and inhibits the activity of the myeloperoxidase enzyme, thus accelerating wound healing. KVV is also an inflammatory process wherein the inoculation of C. albicans in mice also causes a vulvovaginal wound in mice. If prolonged inflammation can cause tissue damage and inhibit wound healing. Propolis accelerates wound healing through its antioxidant and antimicrobial activity. Propolis can also get rid of free radicals that inhibit cell regeneration so that tissue regeneration can take place normally.

This study uses propolis extract from New Zealand with a concentration of ± 0.16% extract per drop. As explained earlier that Propolis is a resin from bees and this depends on the geographical conditions of each place. Study the effectiveness of anti-candida of propolis has been studied by Siti Farida (2019), which examined the in vivo effectiveness of propolis wax derived from bees Tetragonula sp living in Sulawesi-Indonesia and obtained C. albicans can be eliminated by administering it because Propolis Propolis from Sulawesi this contains a polyphenol component that has antioxidant activity, it was also found that the flavonoid content of Indonesian propolis was higher than propolis originating from Romania, Taiwan, Brazil and China. The bioactive components of polyphenols, terpenoid acids, flavonoids have anti-inflammatory, anti-microbial, antioxidant and anti-viral activities.

Mahadewi (2017) also reported that propolis from Indonesia contains anti-fungal marker components such as: thymol, cucumene, tetralline, and Ep-coumaric acid which are potential as anti-candida.

Conclusion

In conclusion, the characteristics of the study subjects were found in all rats with vaginal pH 7-8, all mice used in this study were positively infected with KVV, Estradiol valerate could induce pseudoestrous conditions in Wistar strain mice used in the study. As well as the effectiveness of administration of propolis in mice infected with CVV on day 1,2,3,4 compared to controls found a significant difference with the value of p <0.001 so that it can be concluded that propolis shows a promising therapeutic effect on KKV.

References

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