

A Supplement Based on Zn-Enriched Virgin Coconut Oil as an Antifungal agent for Vaginal Candidiasis Patients

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This research was conducted to investigate the amount of *Candida albicans* in vaginal secretion of Vaginal Candidiasis patients administered with Zn-enriched virgin coconut oil. Thirty respondents were selected based on several criteria as follows: the number of *C. albicans* colonies in the vaginal secretion was more than 10^5 cfu.ml⁻¹, voluntary, healthy, willing to sign the informed consent and resided in Purwokerto. In Group A, 10 women were administered 2 tablespoons per day of Zn-enriched virgin coconut oil. In Group B, 10 women were administered 1 tablespoon per day of Zn-enriched virgin coconut oil; and in Group C, 10 women served as control group. Vaginal secretions were taken 3 times, before intervention (at baseline time), at 1 month and 2 months after intervention. Samples were taken by collecting vaginal secretions from the vaginal proximal region using a sterile cotton bud, which was then put into a tube containing sterile carrier media. The vaginal secretions were tested for the number of total *C. albicans* using Pour Plate Method. Two months after treatment, the number of colonies decreased from 4.4×10^6 to 2.5×10^6 cfu ml⁻¹ ($p=0.03$) in Group A. There was no significant difference in the number of colonies between Group A and Group B, the number of *C. albicans* colonies was still above the normal range. Therefore, the recommended dosage of intervention with Zn-enriched virgin coconut oil is one tablespoon a day.

Key words: *Candida albicans*, vaginal candidiasis, virgin coconut oil, Zn

Candida albicans is a normal microbiota that is commonly found in the vagina. However, excessive amount of this yeast can cause discomfort, pruritis, and pain. This condition is known as leukorrhea or Vaginal Candidiasis (VC). Many conditions can trigger the emergence of VC, such as trichomoniasis, diabetes mellitus, vaginitis senilis, inflammatory chronic inflammation of the pelvis, virus infection, disturbances in immune function, and Candidiasis. The last two conditions were commonly experienced by women (Sobel 2005; Winarsi *et al.* 2006). Transmorphism of *C. albicans* from yeast to mycelia is potentially pathogenic. The mycelium of *C. albicans* is capable of binding to the epithelium of the hosts cells mycelia and penetrating the surface with mycelium protein, which is then tightly cross-linked with the cells epithelium.

Proteins contain amino acids that are able to act as a transaminase substrate of mammalian cell keratin. Binding of *C. albicans* enzymes to the epithelium of host cells leads to pathogenic process. Proteinase and phospholipase secreted by the mycelium are capable of digesting epithelium cells, which then facilitates the invasion by the mycelium. *C. albicans* itself may perform phagocytosis on the endothelium of the host cell. This ability enhances the virulence of *C. albicans*.

Winarsi *et al.* (2006) reported that VC incidence in Purwokerto was 38%, which occurred among women of 15-53 years of age that experienced Zn deficiency (Winarsi *et al.* 2005). Zn is a co-factor for enzyme that has antifungal, antiadenoma, antiprostatitis, and immunostimulator potency. It is important for maturation, activation, proliferation, and differentiation of T-cells. Therefore, the occurrence of VC is likely to be worse in women with Zn deficiency.

The application of clinical medicine might cause immunosuppressor effect. Therefore, people prefer to use

natural remedies such as Virgin Coconut Oil (VCO). VCO not only contains lauric acid, but also capric, caprylic, and myristic acids (Ingle *et al.* 1999). These fatty acids have antifungal, antibacterial, as well as antiviral potency, and they help maintain the immune system (Bergsson *et al.* 2001). Lauric acid can kill *C. albicans* and repair the metabolism energy (Portillo *et al.* 1998).

The aim of this research was to investigate the growth of *C. albicans* in vaginal secretions of VC patients treated with Zn-enriched VCO.

MATERIALS AND METHODS

Zn-enriched VCO has been formulated by Winarsi *et al.* (2006). Thirty VC patients were selected based on the following criteria: number of *C. albicans* in the vaginal secret (more than 10^5 cfu ml⁻¹), resident in Purwokerto, willing to volunteer for the research and sign an informed consent form. Subjects were divided into 3 groups with 10 patients in each group. Those in Group A were treated with 2 tablespoons per day, those in Group B were treated with 1 tablespoon per day, while those in the Group C were given a placebo and served as control group. Treatments were carried out for two months. Vaginal secretion were sampled at three periods: *i.e.* at baseline and then continued by one and two months after treatment. Sample was taken by sweeping the vaginal proximal area using a sterile cotton bud, which was then put in a tube containing sterile carrier media. The sample was then tested for the total number of colonies of *C. albicans* using Pour Plate Method.

RESULTS

The amount of *C. albicans* at baseline time was above the normal range, being 8.38×10^6 - 1.24×10^7 cfu ml⁻¹ (Fig 1). One month after beginning treatment, the number of *C. albicans*

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colonies decreased from 8.4×10^6 to 4.8×10^6 cfu ml⁻¹ ($p = 0.022$) group receiving 2 tablespoons of VCO enriched with Zn per day (Group A). However, the number of colonies remained above normal. There was no significant difference on the amount of *C. albicans* between Group A and Group B ($p = 0.32$). Because the amount of *C. albicans* was still high, the time of treatment was lengthened. After 2 months of intervention, the number of *C. albicans* colonies in Group A decreased from 4.4×10^6 to 2.5×10^6 cfu ml⁻¹ ($p = 0.03$).

The growth of *C. albicans* is influenced by pH. Measurements of pH of vaginal secretions of VC patients are presented in Table 1. The pH at baseline time was relatively neutral approximately 6.0. One month after intervention, the pH decreased from 5.8 to 5.5, and continued to decrease 2 months after treatment, from 5.3 to 5.0 (Table 1).

DISCUSSION

At baseline time, the amount of *C. albicans* was similar among the 3 groups ($p = 0.45$), indicating that the groups were homogeneous before treatment. Therefore, a change in the number of colonies after treatment reflects the effect of Zn-enriched VCO.

VC is a pathological infection condition. One factor causing VC is the excessive amount of *C. albicans* in vaginal secretions. In this case, the host immune system is disrupted by *C. albicans*. This causes rapid proliferation of yeast cells, which results in increasing amount of *C. albicans*.

Fungal cells secrete enzymes that facilitate their invasion. Secreted Aspartyl Proteinase (SAP) produced by *C. albicans* increases the microorganism's ability to colonize and penetrate into the host tissue. In the host's body, the yeast cells are sometimes unrecognized by the host's immune

system (Zeppelin *et al.* 1998). SAP induces the release of mannan (a component of the fungal cell wall), which then inhibits and modulates the host's immune system. This enzyme suppresses the host's immunoglobulin and complement levels (Naglik *et al.* 2003).

SAP hydrolyzes the mucous secreted by the host's digestive tract, so that *Candida* cells may directly penetrate the mucous cells (Chaffin *et al.* 1998). *Candida* cells are still able to secrete SAP even after they have been subjected to phagocytosis by macrophages. Therefore, the activity of *Candida* cells is stronger than what the host's body expected. This situation leads to suppression of the host's immune system and thus causes infection.

Mannoproteins and enolase (metabolites of *C. albicans*) are antigens that are able to stimulate the host's humoral immune response. Thus, mannoprotein modulates the host's immune response. It makes *C. albicans* unrecognized to the host's immune system, so that the cells are not opsonicated or phagocytosized. The other mechanism by which *C. albicans* makes itself unrecognized to the host immune system is through adhesion with components of the host's cells, including thrombocyte, and complement of iC3b. This triggers phospholipase release by *C. albicans*, which assists the penetration to the host's tissue and crushes the cell membrane. The activity of phospholipase is the primary factor affecting virulence in *C. albicans*.

Candidiasis incidence is a consequence of *C. albicans* virulence, which is influenced by the metabolites of *C. albicans* and a predisposition factors of the host's body. Regarding the immunocompetence between the organism and the host's normal immune system, *C. albicans* acts as a normal microbiota on the skin, mucous surfaces, digestive tract, urethra, and genitalia. Accompanied by other normal microbiota, *C. albicans* balances the formation of colonies, so that the growth of the pathogenic microbes may be prevented and a balanced pH is maintained.

In normal amounts, *C. albicans* is not pathogenic, because it can be controlled by the immune system and other normal microbiota. The existence of *C. albicans* and other normal microbiota have a competitive effect, especially on the adhesion and nutrition absorption ability of the host's cells. Under certain conditions, the amount of *C. albicans* cells in the body increases when the activity of immune cells decreases. This condition disturbs the balance among other normal microbiota or other factors which triggers the growth of *C. albicans*. Candidiasis represents an opportunist infection, so that infection usually occurs in immunocompromized individuals (Fridkin and Jarvis 1996).

The decrease in the number of *C. albicans* colonies after 1 month of intervention with Zn-enriched VCO was related to the components of organisms cell walls. Chaffin *et al.* (1998) and Marcilla *et al.* (1998) stated that the cell walls of *C. albicans* were composed of glucan, chitin, mannoprotein (mannan binding to protein), protein, fat, and inorganic salts as minor components. These components build up the yeast cell walls and mycelium in the relatively same amount. Glucan builds up cell structure, while chitin maintains integrity of the cell wall structure (Marcilla *et al.* 1998). Mannoprotein and other proteins are predominant in the external layer of the cell wall with only small amounts being found in the

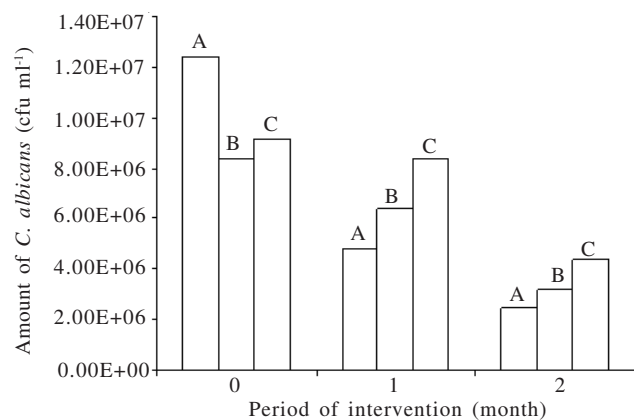


Fig 1 The amount of *Candida albicans* colonies. A, group treated with Zn-enriched VCO, 2 tablespoons/day; B, group treated with Zn-enriched VCO, 1 tablespoon per day; C, control group.

Table 1 The average pH of vaginal secretion

Group	pH in the period of intervention (month)		
	0	1	2
A	6.1	5.8	5.3
B	6.1	5.6	5.1
C	6.0	5.5	5.0

A, group treated with Zn-enriched VCO, 2 tablespoons per day; B, group treated with Zn-enriched VCO, 1 tablespoon per day; C, control group.

internal layer. Mannoprotein is covalently bound to β -glucans and protein chains (Chaffin *et al.* 1998). Mannoprotein is reported to trigger host immune responses to Candidiasis, because this compound is thought to be involved in the changing of cell morphology. Mannoprotein has immunomodulator potency to the host's body. It generally controls the host's immune system, including natural killer, phagocytes (macrophage), as well as cellular and humoral immune cells (Chaffin *et al.* 1998; Marcilla *et al.* 1998). Zn-enriched VCO may attract mannoproteins, the primary component of *C. albicans* exterior cell walls, causing destruction of the cell walls. This will cause glucan and chitin, other components of *C. albicans* cell walls, unable to maintain the integrity of the entire cell walls, thus making the cell walls very brittle. A brittle cell wall can be easily lysed, and unable to maintain *C. albicans* structure. Brittleness of *C. albicans* cell walls may be related to glycoprotein because the ability of the external layer of cell walls to act as an adhesion mediator on host epithelial cell surface is interrupted (Chaffin *et al.* 1998). These conditions could therefore suppress the growth of *C. albicans*.

Goyal and Khuller (1992) reported that the cell membrane of *C. albicans* consisted of a phospholipid layer, which was one of its energy sources. When *C. albicans* is in contact with lauric acid in the host body, the lipid compounds of cell wall will be lysed. As the lipid content of the membrane is destroyed, the cell content leaks out. Therefore, the growth of *C. albicans* is inhibited and the *C. albicans* can even be killed. Caprylic acid derivatives of VCO could also kill VC causing *C. albicans*. The potency of VCO as an antifungal agent is shown by the activity of lauric and caprylic acids. Lauric acid in the body is converted into monolaurine, while caprylic acid is converted into monocapriline compounds. These compounds are monoglycerides which have antimicrobial activities capable of regulating the growth of *C. albicans* (Bergsson *et al.* 2001).

Zn also has an antimicrobial potency, especially towards pathogenic microbes. In the form of free ions, they can directly attack microbes. Zn is also a component of human membrane cell structure and it is an essential antioxidant for cells with short half-life, such as immune cells (Filipe *et al.* 1995). Therefore, Zn fortified by VCO will improve the capacity of the host immune and defense systems.

Specific protein compilers of the cell wall are responsible for *C. albicans* dimorphism (Marcilla *et al.* 1998), but this dimorphism also depends on temperature, pH, and CO₂ concentration (Ernst 2000). *C. albicans* optimally grows at 37°C and neutral pH. At neutral pH (at baseline time), the organism is in a mycelial state, but at lower pH (after 1 and 2 months of treatment) it is in yeast state (Molero *et al.* 1998). In the yeast form, adhesion of fungal cells to the host epithelial cells is stronger.

Protein and glycoprotein components of the *C. albicans* cell wall surface are also important in adhesion (Senet 1998), which is the first step of colonization and infection (Chaffin *et al.* 1998). Adhesion occurs at a minimum pH of 3-4, and is optimal at pH 6 (Sundstrom 2000). At relatively neutral pH (at baseline time), cell colonization could occur, so that the

number of *C. albicans* colonies were higher than normal. At decreased pH, although not significant, the number of *C. albicans* colonies declined.

Kanbe and Cutler (1994) argued that *C. albicans* was capable of producing a lactic acid compound in vaginal secretion. Lactic acid causes the cell wall of *C. albicans*, which consists of mannan, mannoprotein and chitin were denaturated, and reduce the level energy of this microorganism. This finding supports the statement of Klotz and Smith (1995), that some microorganisms are more sensitive to acidic environment. As proteins, enzymes will be denaturated by acid. This causes physiological disturbance and a decrease of the microbe's life-time. A decrease of pH will also improve the inhibition of *C. albicans* growth by VCO.

By the end of the treatment (2 months), the amount of *C. albicans* in the vaginal secret were still above the normal range. A greater effect may be achieved by lengthening the intervention period, so that the normal amount of vaginal microbiota would be recovered. After 2 months of intervention, both dosage given did not show any significant difference ($p = 0.26$). Therefore, based on technical and economical considerations, the recommended dosage is 1 tablespoon of Zn-enriched VCO per day.

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