ISSN: 03875547
Volume 46, Issue 04, April, 2023

Holothurin's Impact on E-cadherin in Wistar-Rat Vaginal Epithelium After Candida Albicans Infection

Sri Handayani[1,2*], Nurdiana[3], Sri Winarsih[4], Agustina Tri Endharti[5]

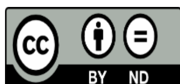
Doctoral Program of Medical Science, Faculty of Medicine, Brawijaya University, Malang, Indonesia[1]
Departement of Midwifery of Aisyiyah Surakarta University[2]
Departement of Microbiology Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia[3]
Departementof Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia[4]
Departementof Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia[5]

Corresponding author: 1,2*

Keywords:
E-cadherin, candida albicans, holothurin, caspofungin, antifungal

ABSTRACT

E-cadherin is a protein that plays a role in cell adhesion and regulates the stability of contact interactions at the cell surface. E-cadherin production may decrease in cases of C. albicans infection, which may affect the integrity and stability of vaginal epithelial cells. A total of 48 rattus norvegius wistar rats were divided into 4 positive control groups (P1) were given topical C. albicans after being grown in yeast extract peptone dextrose (YPD) in the vagina of rattus norvegicus wistar white rats. Groups P2 & P3 were treated with Holothurin 3500 ug and caspofugin 140 ug topically on the vagina of the animal model in 12-, 24- and 48-hours intervals. Immunofluorosens was performed to analyze the results quantitatively using imageJ software and qualitatively by listing the imaging. Data were then processed using SPSS version 23 statistical software. Measurement of E-cadherin expression after VVC for 12, 24, and 48 hours showed no significant difference in all groups. The negative control group had lower E-cadherin expression than the treatment groups, and in the C. albicans positive treatment group, E-cadherin expression increased over time. The holothurin-administered group showed suppression of E-cadherin expression at hours 12 and 24, however at hour 48 E-cadherin expression rose slightly although not significantly. Holothurin given to experimental animals with candidiasis has significant changes in suppressing the amount of E-cadherin in the vaginal epithelial tissue of wistar rattus norvegicus.



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.

1. INTRODUCTION

Vulvovaginitis is an inflammation of the vagina and vulva that can be caused by various factors, including fungal infections, such as Candida albicans. Fungal vulvovaginitis is the most common infection in adult women, affecting about 75% of women at least once in their lifetime. Common symptoms associated with vulvovaginitis include itching, burning, unpleasant odor, and abnormal vaginal discharge [1].

In response to C. albicans invasion vaginal epithelial cells play an important role in determining whether or not the fungus can reproduce. Vaginal epithelial cells form a layer known as the mucosal epithelium, which serves as the first barrier against microbial infection and also plays a role in triggering an immune response against pathogens [2].

E-Cadherin is a mucosal epithelial cell protein that plays an important role in maintaining cell structural integrity and inhibiting pathogen invasion. In addition, E-Cadherin is also involved in interactions between epithelial cells and immune cells that are important in responding to infection [3]. Recent studies have shown that C. albicans is able to decrease E-Cadherin expression on vaginal epithelial cells, thereby increasing the ability of the fungus to invade and multiply on vaginal epithelial cells [4].

Proteolytic degradation of E-Cadherin refers to the breakdown of the E-Cadherin molecule into smaller fragments by protease enzymes. E-Cadherin is a transmembrane protein that plays an important role in maintaining the structural integrity of epithelial cells and inhibiting the invasion of pathogens such as Candida albicans. E-Cadherin consists of an extracellular domain, a transmembrane domain, and an intracellular domain [5].

Several studies have shown that C. albicans invasion of Wistar rat vaginal epithelial cells can decrease the expression of E-Cadherin on vaginal epithelial cells, which may then result in proteolytic degradation of E-Cadherin. Protease enzymes, such as matrix metalloproteinases (MMPs), have been shown to mediate the proteolytic degradation of E-Cadherin in C. albicans-infected vaginal epithelial cells [6].

Proteolytic degradation of E-Cadherin can disrupt the structure and function of mucosal epithelial cells, thereby increasing the ability of C. albicans to invade and multiply on vaginal epithelial cells. In addition, proteolytic degradation of E-Cadherin may also affect the interaction between epithelial cells and immune cells involved in the immune response to infection [7].

Holothurin is a bioactive compound found in sea cucumbers that has been shown to have potential as an antimicrobial and antifungal agent [8]. Several previous studies have shown that holothurin can exhibit antibacterial and antitumor activities, however, research on the ability of holothurin as an antifungal in Candida infection is still limited.

Research on the ability of compounds, such as holothurin, to prevent proteolytic degradation of E-Cadherin on mucosal epithelial cells may provide new insights into the potential of these compounds as therapeutic agents for fungal infections of vaginal epithelial tissues. In this context, studies on the ability of holothurin to relieve C. albicans invasion on E-Cadherin of Wistar rat vaginal epithelium are important.

This study may provide new information on the potential of holothurin as a therapeutic agent for fungal infections of vaginal epithelial tissue. This study may also provide new insights into how holothurin may affect the interaction of mucosal epithelial cells and fungi at the molecular and cellular levels, particularly in the context of the role of E-Cadherin in responding to fungal infections. This information could be useful for the development of new therapies for human fungal infections, including the development of more effective holothurin drugs and formulations.

2. Methods

In this study, the research design was true experimental with a post-test only control group design approach. This research was conducted from January to March 2021 and obtained an ethical permit from the Faculty of

Medicine, Universitas Brawijaya number 337/EC/KEPK-S3/12/2019. A total of 48 female Rattus norvegicus wistar were randomly selected for acclimatization for one week before treatment. The whole sample was randomly divided. Each group consisted of 12 Rattus norvegicus wistar rats Group 1 (K1) was negative control, positive control (C. albicans) (P1), C. albicans + holothurin (P2), C. albicans + caspofungin (P3) and each treatment group was divided by intervention group 3 times (12, 24 and 48 hours). We excluded mice that were born prematurely, and died before treatment. Positive control (P1) was topically treated with C. albicans after growing in yeast extract peptone dextrose (YPD) in the vagina of rattus norvegicus wistar white rats. Groups P2 & P3 were treated with Holothurin 3500 ug and caspofugin 140 ug topically in the vagina of the animal model. Subsequently randomly selected for termination with intraperitoneal ketamine 100 mg before tissue was taken.

2.1 Preparation of C. albicans

C. albicans isolate (4506547065307370) was obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya and inoculated overnight on Sabouraud Dextrose Agar (SDA; Sabouraud Dextrose Broth (Ph 5.6)), Crystal violet, lugol, 96 percent alcohol, aguades, and Dimethyl Sulfoxide (DMSO)). C. albicans cultures were then propagated in Yeast extract Peptone Dextrose (YPD) media and incubated overnight at 37°C in an incubator. C. albicans were harvested and washed in sterile PBS and cell suspension (Sigma Chemical Co., St. Louis, MO, USA). For subsequent analysis, cells were prepared in 0.9 percent NaCL with a cell density of 1.0 105 CFU/mL.

2.2 Examination of E-Cadherin Expression using Immunofluorescence.

The materials used were vaginal tissue slides, labeled primary antibodies [anti-E-cadherin polyclonal antibody (alexa fluor ® 647 conjugated) and rabbit anti-E-cadherin/CD284 polyclonal antibody (alexa fluor 488 conjugated)]. Observation with Fluorescence microscope with 400x magnification. The examination was started by making histology preparations. Then the tissue taken was fixed using neutral buffered formalin (10% solution in water) at room temperature for 24 hours, then dehydrated by putting the fixed tissue into alcohol successively from 70% to 100% expression then cleared by immersing the organ in xylol solution for 24 hours. Infiltration is done by incubating the tissue in liquid paraffin and then put into an incubator at 55oC - 57oC for 12 hours. The next process is embedding to make paraffin blocks by embedding organs in solid paraffin at 20oC - 25oC then cut using a microtome with a thickness of 4 μm. The slices are attached to a glass object smeared with mayers albumin, labeled according to the sample code and then stored in an incubator at 37oC overnight so that they stick strongly, then ready for staining. For immunohistochemical staining, the incision on the glass slide was glued with neofren to avoid detachment of the incision from the glass slide during staining.

After the slide is finished, then perform staining with E-Cadherin antibody The process begins by heating the slide at 60oC for 60 minutes. Then immersed in the solutions below in order: Xylol (2 x 10 minutes), absolute Ethanol (2 x 10 minutes), 90% Ethanol (1 x 5 minutes), 80% Ethanol (1 x 5 minutes), 70% Ethanol (1 x 5 minutes), and sterile Aquades (3 x 5 minutes).

Antigen Retrieval with Citrate Buffer. Slides were immersed in a chamber containing citrate buffer pH 6.0, then placed in a waterbath at 95oC for 20 minutes. Slides were removed from the waterbath, wait until room temperature ± 20 minutes. Side washed with PBS 3x5 minutes. Slides were washed with PBS Triton-X 100 0.1% for 1 x 5 minutes. Incubated with 1% bovine serum albumin (BSA) for 30 minutes at room temperature. BSA solution was discarded. Then incubated with TLR4 labeled primary antibody overnight in 4oC. Slides were washed with PBS 3 x 5 minutes. Incubate with DAPI 1:1000 for 5 minutes. Wash with PBS 3 x 5 minutes. Cover with mounting medium and cover glass. After the preparation is complete, observations are

made with a Fluorescence microscope with a magnification of 400x. Calculation of immunofluorescence result data is done by entering it in ImageJ software. Mean analysis for numerical data using the mean and standard deviation (SD) of each variable with an independent sample t-test on univariate normally distributed data. Statistical analysis using One Way ANOVA test on SPSS 23 software.

3. Result

3.1 E-cadherin expression in the vagina of wistar rats

The potential of holothurin on e-cadherin and the degree of VVC inflammation in wistar rattus norvegicus, with samples in the form of immunofluorosens preparations with the ImageJ method. Data representation can be expressed in Figure 1.

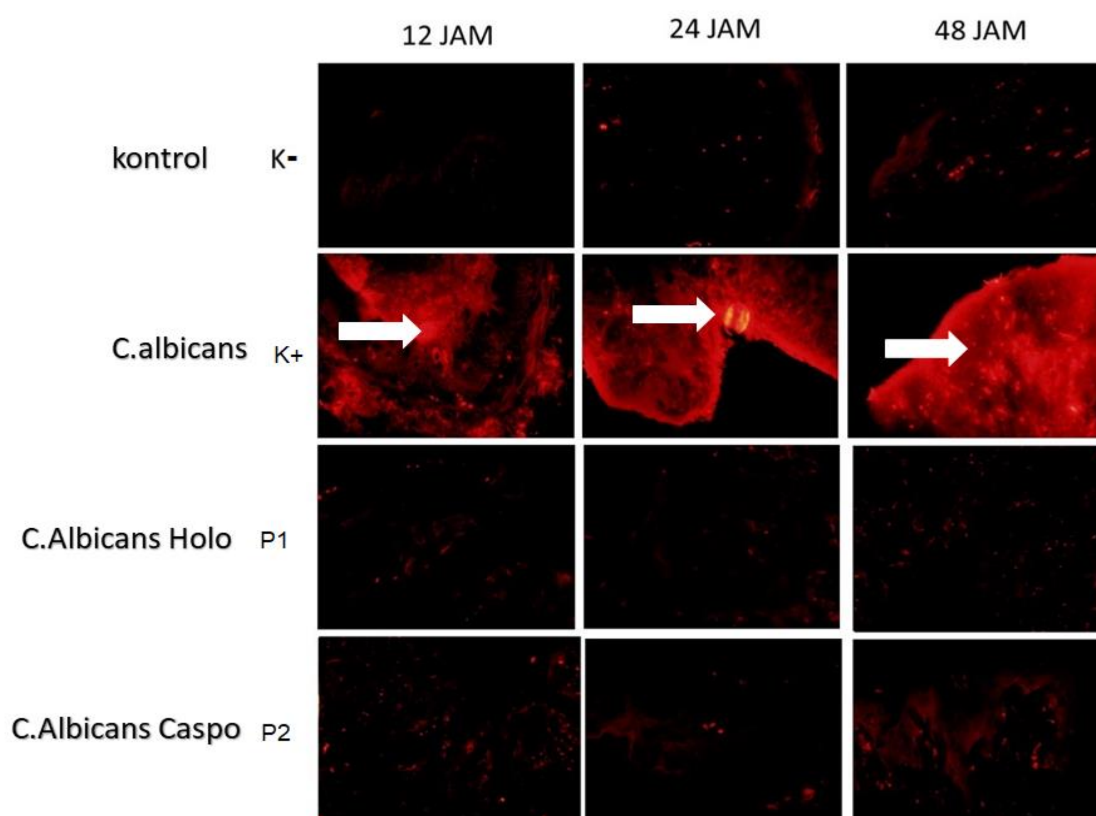


Figure 1 Expression of E-cadherin in the mushroom and active ingredient treatment groups of Holothurin and Caspofungin at 12, 24 and 48 hours in Wistar rats. K-: Control without any treatment (healthy). K+: C. albicans treatment. P1: Fungus/C.albicans treatment administered with Holothurin. P2: Fungus/C.albicans treatment administered with Caspofungin..

E-cadherin expression in the negative control group was lower than in the treatment groups K+, P1, and P2. Furthermore, in the positive treatment group C. albicans (K+) at hour 12 was higher than the K- group, hour 24 was higher than the K- group, hour 48 was higher than the K- group, so it can be said that there was growth of C. albicans. Groups with holothurin and caspofungin administration at 12, 24 and 48 hours, appear to reduce e-cadherin expression when viewed from Figure 1.

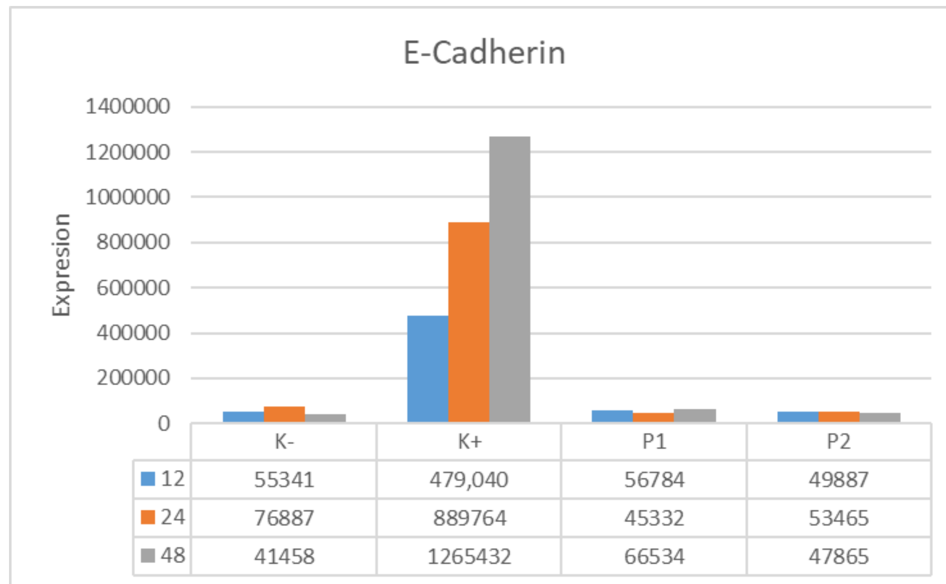


Figure 2 Graph of E-cadherin expression in the mushroom treatment and active ingredient treatment groups of Holothurin and Caspofungin at 12, 24 and 48 hours in Wistar rats. K-: Control without any treatment (healthy). K+: C. albicans treatment. P1: Fungus/C.albicans treatment administered with Holothurin. P2: Fungus/C.albicans treatment administered with Caspofungin.

Furthermore, the analysis of E-cadherin cells at 12 and 24 hours of observation was carried out ANOVA analysis to see if there were significant differences between groups. Then continued with post hoc Bonferroni (homogeneous variety) or Games Howell for non-homogeneous data variety). While at 48 hours observation, Kruskal Wallis + Post Hoc Mann Whitney analysis was performed.

Table 1 Comparison of E-Chaderin 12 Hours

Observation Group	**Mean ± standard deviation**	***p-value ANOVA***	***Post Hoc Games Howell***
Negative control (K-) a	17677,50 ± 6410,65	0,000	a-b (0,259)
Positive Control (K+) b	11838,33 ± 2765,93		**a-c (0,001)**
			a-d (0,009)
Holothurin (P1) c	61547,17 ± 13876,65		**b-c (0,001)**
Caspofungin (P2) d	54826,83 ± 17315,61		**b-d (0,006)**
			c-d (0,878)

Description: If p-value <0.05 means there is a significant difference and if p-value>0.05 means there is no significant difference.

The results of E-Chaderin testing at 12 hours showed that there were significant differences in groups K- and P1 (p=0.001), K- and P2 (p=0.009), K+ and P1 (p=0.001), K+ and P2 (p=0.006).

Table 2 Comparison of E-Chaderin 24 Hours

Observation Group	**Mean ± standard deviation**	***p-value Anova***	***Post Hoc Bonferroni***
negative control (K-) a	43257,00 ± 14699,61	0,000	**a-b (0,017)**

Positive control (K+) b	17195,33 ± 11606,342		**a-c (0,006)**
			a-d (0,874)
Holothurin (P1) c	72663,00 ± 707,10		**b-c (0,000)**
Caspofungin (P2) d	54826,83 ± 17315,61		**b-d (0,000)**
			c-d (0,1818)

Description: If p-value <0.05 means there is a significant difference and if p-value>0.05 means there is no significant difference.

The results of E-Chaderin testing at 24 hours showed that there were significant differences in groups K- and K+ (p=0.017), K- and P1 (p=0.006), K+ and P1 (p=0.000) and K+ and P2 (p=0.000).

Table 3 Comparison of E-Chaderin 48 Hours

Observation Group	Mean ± standard deviation	*p-value Kruskal Wallis*	*Post Hoc Mann Whitney*
Negative control (K-) a	18084,67 ± 4404,07	0,001	a-b (1,000)
Positive control (K+) b	18014,83 ± 9843,93		**a-c (0,004)**
			a-d (0,006)
Holothurin (P1) c	50560,33 ± 11035,35		**b-c (0,004)**
Caspofungin (P2) d	28688,00 ± 10405,44		b-d (0,078)
			c-d (0,016)

Description: If p-value <0.05 means there is a significant difference and if p-value>0.05 means there is no significant difference.

The mann whitney test results of E-Chaderin data at 48 hours showed that there were significant differences in groups K- and P1 (p=0.004), K- and P2 (p=0.006), K+ and P1 (p=0.004) and P1 and P2 (p=0.016).

Table 4 Correlation analysis of E-Cadherin at 12, 24 and 48 hours.

Observation Group	12 hours and 24 hours	12 hours and 48 hours	24 and 48 hours
Negative control (K-)	p = 0,471 r = 0,369	**p = 0,002** **r = 0,968**	p = 0,252 r = 0,556
Positive control (K+)	**p = 0,007** **r = 0,932**	p = 0,122 r = -0,699	p = 0,062 r = -0,789
Holothurin (P1)	p = 0,344 r = 0,472	p = 0,107 r = 0,720	p = 0,919 r = -0,054
Caspofungin (P2)	**p = 0,000** **r = 1,000**	p = 0,854 r = 0,098	p = 0,854 r = 0,098

The correlation results between observations show that there is a significant positive correlation between 12 and 48 hours in the K- treatment. Then there is a significant positive correlation between 12 and 24 hours in the K+ treatment and there is a significant positive correlation between 12 and 24 hours in the P2 treatment.

Table 5 Pearson Correlation Analysis of E-Cadherin comparisons at 12, 24 and 48 hours

TMJ ISSN: 03875547

ISSN: 03875547
Volume 46, Issue 04, April, 2023

		E_Chaderin_12	E_Chaderin_24	E_Chaderin_48
E_Chaderin_12	Pearson Correlation	1	.845**	.732**
	Sig. (2-tailed)		.000	.000
	N		24	24
E_Chaderin_24	Pearson Correlation		1	.598**
	Sig. (2-tailed)			.002
	N			24
E_Chaderin_48	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

**. Correlation is significant at the 0.01 level (2-tailed).

Overall, there is a significant correlation between observation times of 12, 24 and 48 hours with a positive correlation, meaning that the longer the observation time, the higher the E-cadherin expression. The results of measuring E-cadherin expression after experimental animals experienced VVC within 12, 24 and 48 hours in all groups showed no significant difference. E-cadherin expression in the negative control group was lower than the treatment groups K+, P1, and P2.

Furthermore, the C. albicans positive treatment group at hour 12 was higher than group K, hour 24 was higher than group K-, hour 48 was higher than group K-. The group with holothurin administration at hours 12 and 24, appeared to suppress e-cadherin expression. However, at 48 hours, the expression of E-cadherin was seen to increase slightly although it was not significant to the administration of holothurin antifungal.

4. Discussion

This study wanted to evaluate the ability of holothurin compound in relieving Candida albicans invasion on vaginal epithelial cells of Wistar rats through the mechanism of preventing proteolytic degradation of E-Cadherin. The results showed that holothurin could maintain the expression of E-Cadherin on vaginal epithelial cells and reduce the activity of protease enzymes, such as MMP-2 and MMP-9, which play a role in the proteolytic degradation of E-Cadherin. Holothurin showed low expression of E-Cadherin at 12 hours, which was a significant change compared to the K- and P1 groups. E-cadherin is one of the agents associated with host cell protection actin. C. albican decreases the expression and production of e cadherin protein (Rouabhia M, et al. 2012). Increased epithelial infection due to C. albicans induces a decrease in E-cadherin protein at hour 24 and eliminated at hour 48. Holothurin showed low expression of E-Cadherin at hour 24 had a significant change compared to groups K- and K+, K- and P1, K+ and P1, and K+ and P2.

The increase in E-Cadherin expression on vaginal epithelial cells was also followed by a decrease in C. albicans invasion on vaginal epithelial cells treated with holothurin. Therefore, holothurin has potential as a therapeutic agent for fungal infections, especially those caused by C. albicans. However, it should be noted that this study was conducted in Wistar rats, so the results should be interpreted with caution in the human context. These findings are in line with previous studies showing that proteolytic degradation of E-Cadherin plays an important role in the invasion of pathogens, including C. albicans, on mucosal epithelial cells [9].

The various profiles of C. albicans species are pathogens that cause disease is still a matter of debate [10]. The role of epithelial mucosa is an important physical wall to prevent infection due to physical, chemical and microbial agents. The key to candidiasis occurs in the epithelium and penetrates into the connective tissue. C. albicans in the vaginal epithelium of wistar rats against E-cadherin expression. C. albicans is able to invade

epithelial cells through endocytosis and active penetration [6].

E-Cadherin is so massive in the positive control group, this is true because of the invasion of C. albicans, while E-cadherin is expressed when there is extracellular stress. In general, endocytosis of C. albicans is induced from the binding of fungal cells with cell surface proteins such as ALS3p on E-cadherin hosts [11]. However, it turns out that E-cadherin has a specific endocytosis pathway identified as a bonding point between the host and C. albicans. This endocytosis pathway was targeted by antifungal drugs, and the expression of E-cadherin was evaluated in C. albicans invasion events at 12, 24 and 48 hours.

E-cadherin as an epithelial companion acts as an invasin receptor (Als3p) that mediates the induction of endocytosis, and it can happen that E-cadherin decreases C. albicans invasin and inhibits endocytosis [12]. Holothurin showed low expression of E-Cadherin at 48 hours. E-cadherin has an important role in tissue defense due to C. albicans infection, so in this study antifungals in the form of Holothurin and Caspofungin were targeted for testing against C. albicans within 12, 24, and 48 hours.

If E-cadherin is high, tissue damage will not occur so rapidly and massively. If E-cadherin drops due to caspofungin and/or holothurin therapy, it can be concluded that infection does not occur and cells can maintain the actin cytoskeleton so that necrosis does not occur [13].

5. Conclusion

Overall, this study suggests that the compound holothurin has potential as an anti-infective agent in Candida albicans infection of Wistar rat vaginal epithelial cells through the mechanism of preventing proteolytic degradation of E-Cadherin. However, further studies are needed to evaluate the effectiveness of holothurin in humans and understand the mechanisms involved in the detection and prevention of Candida albicans infection in the vagina.

6. References

[1] Mulu W, Yimer M, Zenebe Y, Abera B. Common causes of vaginal infections and antibiotic susceptibility of aerobic bacterial isolates in women of reproductive age attending at Felegehiwot Referral Hospital, Ethiopia: a cross sectional study. BMC Womens Health. 2015 May 13; 15:42. doi: 10.1186/s12905-015-0197-y. PMID: 25968607; PMCID: PMC4438529.

[2] Naglik JR, König A, Hube B, Gaffen SL. Candida albicans-epithelial interactions and induction of mucosal innate immunity. Curr Opin Microbiol. 2017 Dec; 40:104-112. doi: 10.1016/j.mib.2017.10.030. Epub 2017 Nov 17. PMID: 29156234; PMCID: PMC5733685.

[3] Devaux CA, Mezouar S, Mege JL. The E-Cadherin Cleavage Associated to Pathogenic Bacteria Infections Can Favor Bacterial Invasion and Transmigration, Dysregulation of the Immune Response and Cancer Induction in Humans. Front Microbiol. 2019 Nov 8; 10:2598. doi: 10.3389/fmicb.2019.02598. PMID: 31781079; PMCID: PMC6857109.

[4] Richardson, J.P.; Ho, J.; Naglik, J.R. Candida–Epithelial Interactions. J. Fungi 2018, 4, 22. https://doi.org/10.3390/jof4010022

[5] Huguenin M, Müller EJ, Trachsel-Rösmann S, Oneda B, Ambort D, Sterchi EE, Lottaz D. The metalloprotease meprinbeta processes E-cadherin and weakens intercellular adhesion. PLoS One. 2008 May 14;3(5):e2153. doi: 10.1371/journal.pone.0002153. PMID: 18478055; PMCID: PMC2359857.

[6] Basmaciyan L, Bon F, Paradis T, Lapaquette P, Dalle F. "Candida Albicans Interactions With The Host: Crossing The Intestinal Epithelial Barrier". Tissue Barriers. 2019;7(2):1612661. doi: 10.1080/21688370.2019.1612661. Epub 2019 Jun 12. PMID: 31189436; PMCID: PMC6619947.

[7] Dash, S, Duraivelan, K, Samanta, D. Cadherin-mediated host–pathogen interactions. Cellular Microbiology. 2021; 23: e13316. https://doi.org/10.1111/cmi.13316.

[8] Khotimchenko Y. Pharmacological Potential of Sea Cucumbers. Int J Mol Sci. 2018 May 2;19(5):1342. doi: 10.3390/ijms19051342. PMID: 29724051; PMCID: PMC5983632.

[9] Villar CC, Kashleva H, Nobile CJ, Mitchell AP, Dongari-Bagtzoglou A. Mucosal tissue invasion by Candida albicans is associated with E-cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. Infect Immun. 2007 May;75(5):2126-35. doi: 10.1128/IAI.00054-07. Epub 2007 Mar 5. PMID: 17339363; PMCID: PMC1865768.

[10] Turner SA, Butler G. The Candida pathogenic species complex. Cold Spring Harb Perspect Med. 2014 Sep 2;4(9): a019778. doi: 10.1101/cshperspect. a019778. PMID: 25183855; PMCID: PMC4143104.

[11] Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013 Feb 15;4(2):119-28. doi: 10.4161/viru.22913. Epub 2013 Jan 9. PMID: 23302789; PMCID: PMC3654610.

[12] Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, Ibrahim AS, Edwards JE Jr, Filler SG. Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol. 2007 Mar;5(3):e64. doi: 10.1371/journal.pbio.0050064. PMID: 17311474; PMCID: PMC1802757.

[13] Ayollo DV, Zhitnyak IY, Vasiliev JM, Gloushankova NA. Rearrangements of the actin cytoskeleton and E-cadherin-based adherens junctions caused by neoplasic transformation change cell-cell interactions. PLoS One. 2009 Nov 30;4(11):e8027. doi: 10.1371/journal.pone.0008027. PMID: 19956566; PMCID: PMC2779654.