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Original Article

ANTIFUNGAL EFFECT OF *NIGELLA SATIVA* EXTRACT ON FEMALE WISTAR RATS VULVOVAGINAL CANDIDIASIS MODEL

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ABSTRACT

Objective: To assess the antifungal effect of *Nigella sativa* extract on female Wistar rats vulvovaginal candidiasis.

Methods: This was an analytic study that assess the therapeutic effect of *Nigella sativa* in rats' vulvovaginal candidiasis model. The subjects were 28 rats that had been inoculated with *Candida albicans* and divided into 4 groups: control group (G1), fluconazole group (G2), *Nigella sativa* group (G3) and combination of *Nigella sativa* with fluconazole group (G4). *Candida albicans* colony was measured to assess the therapeutic effect of the treatment.

Results: There were no difference number of *Candida albicans* colony between all group before inoculation (p = 0,274) and after inoculation (p = 0,323). There were a significant decreased number of *Candida albicans* colony on the 72 h after the treatment between the three types of treatment with the control group (*Nigella sativa* group p = 0,002; Fluconazole group p = 0,001; *Nigella sativa*+fluconazole p = 0,001).

Conclusion: *Nigella sativa* has a potential antifungal effect by reducing the number of *Candida albicans* colonies.

Keywords: Nigella sativa, Antifungal, Candida albicans, Fungal inoculation, Pseudoestrus, Vulvovaginal candidiasis, Fluconazole

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INTRODUCTION

Candida species are one of the commonest causes of vaginitis in healthy women of reproductive age. Vulvovaginal candidiasis (VVC) is characterized by vulvovaginal itching, redness and discharge. *Candida albicans* is the most common fungal infection of humans, causing Candidiasis [1].

Complications of vulvovaginalis candidiasis (VVC) that often occur is a pelvic inflammatory disease which can ultimately cause infertility in sexually active women and chorioamnionitis which leading miscarriage or premature birth in women who are pregnant. Immediate and proper management of VVC must be done to prevent complications [2, 3].

Nigella sativa is a member of the Ranunculaceae family and has been used as a natural food and medicine which grows in Asia and the Middle East [4]. It contains Thymoquinone, Thymohydroquinone, Dithymoquinone and Thymol. Thymoquinone inhibits the oxygen cycle as an inflammatory balance process with lipo-oxygenizes. *Nigella sativa* has pharmacological effects as antimicrobial, anti-inflammatory, immune stimulation and anti-cancer properties [5, 6].

The inhibitory effect of fungal by *Nigella sativa* extract has been proved by a study both *in vitro* and *in vivo* against some pathogenic fungal such as *Candida albicans*, dermatophytes, non-dermatophytes and some aflatoxin-producing fungi. It had shown that *Nigella sativa* had a high inhibitory effect on candidiasis in rats *in vivo* and can reduce the number of *Candida albicans* 5-fold in the kidneys, 8-fold in the liver and 11-fold in the spleen. The antifungal effect of *Nigella sativa* treatment for a single day on rats that had been inoculated with the *Candida albicans* apparently inhibited the growth of this pathogen [7, 8].

Further research is needed to evaluate the antifungal activity of Nigella sativa *in vivo,* especially in the vulvovaginal candidiasis model [9].

MATERIALS AND METHODS

Methods

This experimental research was conducted in July-October 2019 on Animal House-Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara to asses the antifungal effect of *Nigella sativa* extract on vulvovaginal candidiasis. Ethical clearance is obtained from Animal House-Faculty of Mathematics and Natural Sciences, Sumatera Utara University.

This study use 28 female Wistar rats (*Rattis norvegicus sp*) aged 2-3 mo with a weight range of 200-250 grams that had fulfilled the inclusion and exclusion criteria. Before we do inoculation, we give estradiol valerate 2 mg subcutaneously intraperitoneal 3 d before inoculation and 4 d after inoculation to make the rat in pseudoestrus state that needed to maintenance the *Candida albicans*, and prevent self-healing.

The breeding of *Candida albicans* was held in the microbiology laboratory, Universitas Sumatera Utara General Hospital. By swapping the vagina with a cotton swab dipped in ATCC 14503 contained 3 McFarland *Candida albicans* cells, the inoculation was done.

The *Nigella sativa* extract was given from the pharmacological laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, using Sodium Carboxymethyl cellulose (Cmc Na) in order to obtain the extract of *Nigella sativa* 5 mg/ml.

The samples were divided into 4 groups: 7 rats in the control group, 7 rats were given *Nigella sativa* extract 5 mg/ ml with a dose of 6.6 ml/kg of body weight, 7 rats were given 10 mg/kg of body weight of fluconazole, and 7 rats were given a combination of *Nigella sativa* extract 6.6 ml/kg of body weight and fluconazole 10 mg/kg of body weight. The treatment was given for 72 h after 24 h inoculation. The colonies of *Candida albicans* on the rat's vagina were measured before inoculation and 24 h after therapy. Vaginal smear samples were obtained, incubated for 48 h on temperature 37 °C and counted in the microbiology laboratory of the Universitas Sumatera Utara Hospital.

Data analyzed was using SPSS 22. We used Kruskal Wallis to assess differences in the four groups, T independent test and Mann Whitney, to assess differences in the number of *Candida albicans* colonies in all four groups.

RESULTS

Table 1: Colonies count of *Candida albicans* before inoculation

Group	n	Colonies count of *Candida albicans*, CFU/Plate				p
		Mean	SD	Median	Min–Max	
G1	7	0,14	0,38	0	1–1	0,274[a]
G2	7	10	18,68	0	0–51	
G3	7	2,14	2,48	2	0–6	
G4	7	0,57	0,79	0	0–2	

[a]Kruskal Wallis, Note: G1 = control group, G2 = fluconazole group, G3 = Nigella sativa group, G4 = fluconazole+Nigella sativa group

The highest mean of the number of *Candida albicans* colonies was found on the fluconazole group with 10+18,68 CFU/plate and lowest mean on the control group with 0,14+0,38 CFU/plate. There was no difference of mean number colonies between four groups before inoculation with p = 0,274.

After the inoculation, the remeasurement of vaginal colonies was done. The highest number of *Candida albicans* colonies was found on the control group with 237,86+106,46 CFU/plate and the lowest fungal colonies were in *Nigella sativa* group with 164,57+124,69. There was no difference of mean number colonies between four groups after inoculation with p = 0,323.

After 72 h treatment, the therapeutic effect measured, we found that the lowest number of *Candida albicans* colonies belong to the combination group with 0,14+0,38 CFU/plate, meanwhile, the highest was in the control group with 130,43+117,76 CFU/plate. There was a significant difference of the mean number of colonies between four groups after 72 h of treatment (p<0,001). From Post Hoc Test, it was seen that there were significant mean differences between Control group and three treatment groups (p<0,05)

Table 2: Colonies count of *Candida albicans* after inoculation

Group	n	Colonies Count, CFU/Plate				p
		Mean	SD	Median	Min–Max	
G1	7	237,86	106,46	300	68-300	0,323[a]
G2	7	136,86	115,77	78	28-300	
G3	7	164,57	124,69	66	60-300	
G4	7	210	95,67	243	56-300	

[a]Kruskal Wallis, Note: G1 = control group, G2 = fluconazole group, G3 = Nigella sativa group, G4 = fluconazole+Nigella sativa group

Table 3: Colonies number of *Candida albicans* after 72 h treatment

Group	n	Colonies number, CFU/Plate				p	Post hoc		
		Mean	SD	Median	Min-Max		G2	G3	G4
G1	7	130,43	117,76	62	43–300	<0,001[a]	0,001[b]	0,002[b]	0,001[b]
G2	7	0,43	1,13	0	0–3			0,101[b]	0,917[c]
G3	7	1,14	0,9	1	0–2				0,027[b]
G4	7	0,14	0,38	0	0–1				

[a]Kruskal Wallis, [b]Mann Whitney, [c]T Independent, Note: G1 = control group, G2 = fluconazole group, G3 = Nigella sativa group, G4 = fluconazole+Nigella sativa group

DISCUSSION

The environment changing of vaginal candidiasis will quickly recover so that the infection will heal quickly without creating a pseudoestrus situation [10]. To create, support and maintenance pathogens for vulvovaginalis candidiasis rat model, special treatment was needed by giving estradiol valerate 2-5 mg subcutaneously intraperitoneal 3 d before inoculation and 4 d after to decrease rat immunity or creating a pseudoestrus situation. If needed, it can be repeated weekly [11, 12].

The number of *Candida albicans* fungal colonies in all groups was increased (Control group: 0,14+0,38 vs 237,86+106,46, Fluconazole: 10+18,68 vs 136,86+115,77, *Nigella sativa*: 2,14+2,48 vs 164,57+124,69; Combination group: 0,57+0,79 vs 210+95,67). This indicated that the rats successfully became vulvovaginalis candidiasis model.

Nigella sativa Linn, Ranunculaceae family, was grown in many parts of the world, especially the Mediterranean region, North Africa, the Middle East and parts of Asia and used by herbal therapy for many diseases [7].

The component of *Nigella sativa* was thymoquinquinone (TQ), alcaloid (nigellicines and nigelledine), saponins (alpha-hederin), flavonoids, proteins, fatty acids, and many others, which have various therapeutic effects [13].

Nigella sativa extract has the strongest antifungal effect against various pathogenic fungal strains, including methanol, ethanol and chloroform extracts [6]. Even water extracts from *Nigella sativa* seeds showed an inhibitory effect on candidiasis in rat [14, 15]. From the results of this study, the administration of *Nigella sativa* extract with Sodium Carboxymethyl cellulose can reduce the number of vaginal colonies of the *Candida albicans* (p = 0,002).

Based on the treatment groups comparison, all the three treatment groups include were fluconazole group, *Nigella sativa* extract group and combination of *Nigella sativa* and fluconazole extract group can reduce the number of fungal colonies compared with the control group (p = 0,001; p = 0,002; p = 0,001).

CONCLUSION

Nigella sativa has a potential antifungal effect by reducing the number of *Candida albicans* colonies after 72 h administration.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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