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Therapeutic Effect of 48 h after *Nigella sativa* Extract Administration on Female Wistar Rats Vaginal Candidiasis Model: An Experimental Study

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Abstract

BACKGROUND: *Candida albicans* was the common causes of vulvovaginalis candidiasis (VVC) in human. To avoid complications, prompt and proper treatment of VVC must be performed. The pharmacological effects of Nigella sativa include antimicrobial, anti-inflammatory, immune stimulation, and anti-cancer properties.

AIM: *N. sativa* has been shown to have an *in vivo* antifungal effect and the purpose of this study was to determine the antifungal and potential *in vivo* therapeutic effects.

METHODS: This research was an empirical study which evaluated the therapeutic effect of the vaginal candidiasis model of *N. sativa* in rats. The subjects were 28 rats inoculated with *C. albicans* and were divided into four groups: Control group (G1), fluconazole group (G2), *N. sativa* group (G3), and *N. sativa* and fluconazole group combinations (G4). The colony of *C. albicans* was assessed to determine the treatment's therapeutic effect.

RESULTS: There was no difference in the number of colonies of *C. albicans* between all the pre-inoculation ($p = 0.274$) and post-inoculation ($p = 0.323$) classes. A substantial decrease in the number of *C. albicans* colonies within 48 h of treatment was observed between the three control group treatment forms (*N. sativa* group $p = 0.046$; fluconazole group $p = 0.002$; and *N. sativa* + fluconazole group $p = 0.002$).

CONCLUSIONS: The therapeutic effect of *N. sativa* has been achieved by reducing the number of colonies of *C. albicans*.

Background

The most common human fungal infection causing candidiasis is *Candida albicans* [1]. The often occurring complication of vulvovaginalis candidiasis (VVC) is a pelvic inflammatory disorder that can eventually cause infertility in sexually active women and chorioamnionitis that leads to miscarriage or premature birth in pregnant women. To avoid complications, immediate and careful control of VVC must be done [2], [3].

A part of the Ranunculaceae family is *Nigella sativa*. It has been used in Asia and the Middle East as a natural food and medicine [4]. It includes thymoquinone, thymohydroquinone, dithymoquinone, and thymol. Thymoquinone inhibits the oxygen cycle as a lipo-oxygenizing inflammatory balance mechanism. The pharmacological effects of *N. sativa* include antimicrobial, anti-inflammatory, immune stimulation, and anti-cancer properties [5], [6].

An *in vitro* and *in vivo* analysis against certain pathogenic fungi such as *C. albicans*, dermatophytes, non-dermatophytes and some aflatoxin-producing fungi has demonstrated the inhibitory activity of fungal *N. sativa* extract. *In vivo*, *N. sativa* has been shown to have a high inhibitory effect on candidiasis in rats and can reduce the number of *C. albicans* 5 times in the kidneys, 8 times in the liver, and 11 times in the spleen. The antifungal effect of single-day *N. sativa* treatment on rats inoculated with *C. albicans* inhibited this pathogen's growth [7], [8].

In rats with vaginal candidiasis, no *in vivo* studies have been reported. To evaluate the antifungal activity of *N. sativa* in vaginal candidiasis, further research is required. To determine the minimum inhibitory concentration and minimum fungicidal concentration of *N. sativa* in *C. albicans* pathogen, Asdadi conducted the analysis [9].

Methods

This was laboratory study to test the therapeutic impact on vulvovaginal candidiasis of *N. sativa* extract.

This study was conducted at Animal House – Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, from July to October 2019. Animal House – Faculty of Mathematics and Natural Sciences, Sumatera Utara University, has received ethical clearance.

This study used 28 female Wistar rats (*Rattus norvegicus* sp.) aged 2–3 months with a 101–240 g weight range that met the requirements for inclusion and exclusion. Before inoculation, 3 days before inoculation, and 4 days after inoculation, we give estradiol valerate 2 mg subcutaneously intraperitoneally to make the rat in the pseudoestrus condition required to preserve *C. albicans* and prevent self-healing.

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

The extract of *N. sativa* was obtained from the pharmacological laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara, using a solvent called sodium carboxymethylcellulose (Cmc Na) to obtain 5 mg/mL of *N. sativa* extract.

The samples were divided into four groups: Seven rats in the control group, seven rats were given 5 mg/mL of *N. sativa* extract at a dose of 6.6 ml/kg body weight, seven rats were given 10 mg/kg body weight of fluconazole, and seven rats were given 6.6 ml/kg body weight of *N. sativa* extract and 10 mg/kg body weight of fluconazole.

The therapy was performed for 48 h after 24 h of inoculation. The rat vaginal colonies of *C. albicans* were assessed before inoculation and 24 h after therapy. Vaginal smear samples were collected, incubated at a temperature of 37°C for 48 h, and counted at the Universitas Sumatera Utara Hospital microbiology laboratory.

The analyzed data used SPSS 22. In the four groups, we used Kruskal–Wallis to evaluate differences, independent t-test and Mann–Whitney to evaluate differences in the number of colonies of *C. albicans* in all four groups.

Results

The highest mean of the number of colonies of *C. albicans* was found in the fluconazole group with 10+18.68 CFU/plate and the lowest mean of 0.14+0.38 CFU/plate in the control group. There was no difference between the mean number of colonies between the four groups with p = 0.274 before inoculation (Table 1).

Remeasurement of vaginal colonies was performed after the inoculation. The highest number of colonies of *C. albicans* was identified with 237.86+106.46 CFU/plate in the control group and the lowest colonies of fungi were 164.57+124.69 in *N. sativa* group. There was no difference between the mean number of colonies between the four groups following inoculation with p = 0.323 (Table 2).

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. G1 = control group, G2 = fluconazole group, G3 = *Nigella sativa* group, G4 = fluconazole + *Nigella sativa* group.

The therapeutic effect calculated after 48 h of treatment was that the lowest number of *C. albicans* colonies belonged to the fluconazole group with 0.14+0.38 CFU/plate, while the highest was 110.29+131.48 CFU/plate in the control group. The mean number of colonies was significantly different between the four groups after 48 h of treatment (p < 0.001). The *post hoc* test showed that substantial mean differences (p < 0.05) were observed between each party. The mean number of fungal colonies in the G2 and G4 groups alone did not indicate major differences (p = 0.476) (Table 2).

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. G1 = control group, G2 = fluconazole group, G3 = *Nigella sativa* group, G4 = fluconazole + *Nigella sativa* group.

Discussion

To produce, support, and maintain pathogens for the rat model of VVC, special treatment was required. Estradiol valerate 2–5 mg was administered intraperitoneally subcutaneously 3 days before inoculation and 4 days after inoculation to reduce rat immunity or to establish a pseudoestrus condition. It can be repeated weekly if needed [10], [11].

Table 3: Colonies number of *Candida albicans* after 48 h treatment

Group	n	Colonies number, CFU/plate				p	Post hoc		
		Mean	SD	Median	Min-Max		G2	G3	G4
G1	7	110.29	131.48	51	9–300	<0.001[a]	0.001[b]	0.006[b]	0.005[b]
G2	7	0.14	0.38	0	0–1			0.001[b]	0.476[c]
G3	7	6.43	5.29	5	1–15				0.005[b]
G4	7	0.57	1.13	0	0–3				

[a]Kruskal–Wallis, [b]Mann–Whitney, [c]T independent. G1 = control group, G2 = fluconazole group, G3 = *Nigella sativa* group, G4 = fluconazole + *Nigella sativa* group.

The evolving environment of vaginal candidiasis will recover rapidly so that without developing a pseudoestrus condition, the infection will heal quickly [12]. The number of fungal colonies of *C. albicans* was increased in all groups (control group: 0.14 + 0.38 vs. 237.86 + 106.46, fluconazole: 10 + 18.68 vs. 136.86 + 115.77, *N. sativa*: 2.14 + 2.48 vs. 164.57 + 124.69; and combination group: 0.57+0.79

vs. 210+95.67). This showed that the rats successfully became models of VVC.

N. sativa Linn., a family of Ranunculaceae, has been cultivated in many parts of the world, especially in the Mediterranean region, North Africa, the Middle East, and parts of Asia, and has been used for many diseases in herbal therapy [7]

Thymoquinone, alkaloid (nigellicine and nigellidine), saponins (alpha-hederin), flavonoids, proteins, fatty acids, and several others that have particular therapeutic effects were the components of *N. sativa* [13].

The most potent antifungal action of *N. sativa* extract has been shown against various pathogenic fungal strains, including methanol, ethanol, and chloroform extracts [6]. Even water extracts from *N. sativa* seeds showed an inhibitory effect on rat candidiasis [14]. The administration of *N. sativa* extract with sodium carboxymethylcellulose will reduce the amount of vaginal cellulose.

In a previous study on systemic candidiasis, the therapeutic effect of *N. sativa* may be decreased by 5-fold in the number of candida species in the kidney, 8-fold in the liver, and 11-fold in the spleen when observed 24 h after administration [14].

The three treatment groups include the fluconazole group, *N. sativa* extract group, and the combination of *N. sativa* and fluconazole extract groups may reduce the number of fungal colonies relative to the control group ($p = 0.001$; $p = 0.006$; and $p = 0.005$), based on a comparison of the treatment groups.

The limitation of this study is that further research is needed to give *N. sativa* with a longer time to see if there is a better therapeutic effect.

Conclusions

We concluded that *N. sativa* has therapeutic effect on VVC that reduces the number of *C. albicans* colonies after 48 h administration.

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