



Effect of Vaginal Candidiasis and Treatments With Oils of Thyme and Oregano Compared With Nystatin on Blood Parameters of Mature Female Rabbits

KEYWORDS

candidiasis , thyme oil, oregano oil, RBC, WBC ,platelets.

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ABSTRACT

Oils of thyme and oregano were extracted by hexane and analyzed to the component in a manner GC-MAS (Gas chromatography). In vitro study was conducted to find the best oily concentration capable of inhibiting growth of candida albicans culture on sabaroud medium. in vivo study was done on seventy two healthy mature non pregnant female rabbits. Animals were divided into nine groups of eight animals each. The first group was injected by (0.1ml) physiological saline vaginally and served as a negative control group. All other eight groups were immune suppressed by drenching each animal with (5 mg/kg) dexamethasone once daily for 7 days,(Group 2) considered as a dexamethasone control group. Other seven groups were infected vaginally with Candida albicans by $(1.5) \times 10^8$ candida cells/ml once daily for 7 days (Group 3) considered as vaginal candida (positive) control. The sixth groups then treated once daily for 14 days with (0.1) ml of oils according to each group. (Group 4) was treated with thyme oil orally, (Group5) treated with thyme oil vaginally ,(Group 6) treated with oregano oil orally,(Group 7) treated with oregano oil vaginally,(Group8)treated oral nystatin,(Group9) treated vaginally with nystatin. Blood was drawn from animals at the end of the experiment. Result conducted to experimental vaginal candidiasis(EVC) did not has any effect on RBCs so as treatment with thyme and oregano oils while RBCs significantly decreased with treatment nystatin by two routes. Hb decreased with (EVC) and only treatment with vaginal thyme oil raised Hb to normal value. PCV did not change in all groups of study. Total WBCs decreased in (EVC) and all treatment groups did not improve this effect except oral thyme oil treatment. Increased lymphocytes in (EVC), thyme oil and oregano oil orally than control. significant decrease in granulocyte in all studies groups.(EVC) did not affect platelets count however oral(thyme or oregano)oil and vaginal nystatin caused decreased in platelets count contrary to the increase platelets in vaginal (thyme or oregano)oil and oral nystatin. MCV increased in vaginal nystatin and decreased in oral nystatin while MCHC increased in vaginal thyme and decrease in vaginal nystatin

INTRODUCTION

Vaginitis is a complicated disease , many factors induce the infection such as virulent strain of candida, antibiotics and steroid caused immune suppression(1) . Vulvovaginal candidiasis (VVC) are caused by a type of yeast called Candida albicans. As candida organism has been recorded to develop a resistance against the classical used antifungal drugs so there is need to search of a new antifungal agents (2,3,4) essential oils have the effective following benefits: such as antifungal properties ;antiparasitic; anti-inflammatory; antioxidant; antimutogenic ,antiseptic, antibacterial and anticarcinogenic and topical analgesies(5). Several studies have insisted on the antifungal characteristics of medical plants essential oils (6) all of them documented that essential oils of medical plants contains many active phytochemicals such as flavonoids; terpenoids; carotenoids; coumarins and curcumin. Thymus vulgaris and origanum vulgare a medicinal herbs belongs to the family Lamiaceae which is largely cultured in several parts of the world .The important active components of their essential oil are carvacrol and thymol which have antifungal; antimicrobial and antioxidant effects (7).Investigated the differences in chemical structures on mechanism of action of essential oils(8). Both thyme oil and oregano oil are capable to destroy the integration and function of the fungal cell membrane. (9) study the physiological effect of Candida albicans on blood and showed that decreased in number of RBCs ,increased in WBCs while Hb and PCV did not change. (10) indicated a decrease in total leukocytes count by means of leucogram test in all ex-

perimental groups this decrease is caused by a decrease in lymphocytes caused by administration of cortisone which cause decreased immunity necessary for induction of experimental candidiasis. In mice experimental infection with Candida albicans PMNs are often noticed in the vagina but this presence has no correlation with infected animals (11). Below normal physiological standards of haematocrits and haemoglobin were shown when different concentrations of extracted thyme oil and emulsified were studied, thus they constitute a case of anemia (12). They found that platelets accumulation caused by collagen can be efficiently decreased by administration thyme .Oregano displayed anti-platelet properties in human plasma by inhibiting arachidonate cascade and thrombin activity

The aims of this study is to determine the ameliorative effects of two traditional herbs oils (thyme and oregano) by two routs of administration on hematological picture after experimental vaginal candidiasis and comparing those effects with effects resulted from treatment by nystatin.

MATERIALS AND METHODS**Preparation of oils Extracts**

The dry leaves of Thymus vulgaris (thyme) ,Origanum vulgare (oregano) were available from Basra Province /Iraq. leaves were cleaned and grounded used for extraction. samples were defatted in a soxhlet apparatus, dissolved by hexane for (3 hr) (13) . separated oil extracted from solvent by using a rotary evaporator at 50°C for one hr. put oil extracted in an oven at 40°C over night then keeping oil

in dark sterile glass container in refrigerator at 4°C.

Chemical composition of oils extracted.

Essential oil of each plant was analyzed in a gas chromatography equipped in Iraq - Basra : University of Basra ; Agriculture College GC_ MS Lab by models SHIMADZU GC MS-QP 2010 Ultra. From the GC-mass analysis the main phenolic compounds contrabuted between thyme oil and origanm oil are: methyl-5-(1-methylethyl) ; Carvacrol ; p-Cymen ; Antioxine ; Isothymol; Karvakrol; 2-Hydroxy-p-cymeneand2-Methyl-5- isopropylphenol

Animals preparation and management

Seventy-two healthy mature non pregnant female domestic rabbits (*Lepus cuniculus*) brought from the local markets / Basra, weighing (1400-1800) grams body weight each. Kept for a four week in the animal house of College of Veterinary Medicine / University of Basra, to be acclimatized Before using the rabbits for the experiment. They were maintained on unrestricted supplies of food that consist of alfa alfa concentrated pellets and water *ad libitum*

Prepared yeast suspension (Inoculum)

Candida albicans isolated from vaginal mucous mem brane of female cows isolated. Inoculums were prepared in concentration of 1.5×10^8 cells/ml by comparative with MacFarland solusion. Well diffusion method used to determine the antifungal activity by mesurment of inhibition zone of (thymeandoregano) oil compared with Nystatin (14,15,16)

Experimental design:

After acclimatization period animal divided in to (9) groups each groups had (8) female rabbits:-

Group 1:- (-ve) control group.

Group 2:- given 5mg/kg dexamethasone orally for (7) days as control group.

Group 3:- given (5 mg/kg) dexamethason orally for 7 days followed by infection with a vaginal candidiasis for 7 days as(+ve control).

Other group treated like G3 plus treating vaginal candidiasis by(0.1ml) dose of oils for 14 days accordingly:

Group 4:- vaginal candidiasis treated with crude(0.1ml) thyme oil orallyfor 14 days.

Group 5:-vaginal candidiasis treated with crude(0.1) thyme oil (vaginal douching) for 14 days.

Group 6:-treated with crude (0.1 ml)oregano oil (orally)for 14days.

Group 7:- treated with crude (0.1 ml) oregano oil (vaginal douching) for 14days.

Group 8:- treated with (0.1 ml) nystatin (orally) for 14days.

Group 9:- treated with (0.1 ml) nystatin (vaginal douching) for 14days. After 24 hour from the last administration (2.5 ml) of blood is collected from the heart put in EDTA tests tube for the hematological analysis.

HEMATOLOGICAL TEST

RBCs count, Hb, PCV, WBCs count, platelets count and differential leukocytic count were measured by using Hematological auto analyzer instrument Genex60- USA.

STATISTICAL ANALYSIS

The results were expressed as mean \pm SE. The comparisons between groups were performed with analysis of

variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences). $P < 0.05$ was considered to be least limit of significance. Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS.

RESULTS

Effects of vaginal candidiasis and treatment with oils of thyme and oregano compared to Nystatin on RBCs, Hb and PCV of mature female rabbits.

it is clear that only treatment with oral nystatin caused significant decrease($p < 0.05$) of RBC count when compared with control and all treatment groups. Also the results of PCV in the table indicate no significant change in (EVC) and all treatment groups. From table(1) also we noticed that candidiasis significantly decreases Hb. SO, treatments with all treatment groups did not improve Hb except treatment with vaginal thyme.

In table (2) it is obvious that (EVC) significantly ($p < 0.05$) decreases WBC count as well as treatments with oil of oregano either oral or vaginal and also treatment with nystatin either oral or vaginal but results of treatments with oil of thyme either oral or vaginal increases WBC count significantly till it reaches the level of control. Also the table clearly indicate that vaginal candidiasis significantly ($p < 0.05$) increases lymphocytes and significantly decreased granulocytes. But treatments with vaginal oregano oil and nystatin either oral or vaginal increases lymphocytes significantly($p < 0.05$) more than controls. In all treatments decreases granulocytes significantly at ($p < 0.05$) compared to controls and (EVC).

Table (3) indicates that candida infection did not change platelets count compared with control however oral (thyme or oregano) and vaginal nystatin treatment caused significant decreased in platelets count than control and candidiasis while vaginal (thyme or oregano) and oral nystatin caused increased in platelets count. In case of MCV results in the table shows no significant effect of candidiasis with all oil treatments but there are significant decrease in MCV when treated with oral nystatin and significant increase when treated with vaginal nystatin. Measurements of MCH in this table shows no significant effect of all studied groups. increase of MCHC measurements results showed no significant change caused by candidiasis neither with control nor with all oil treatments. The only significant difference is with vaginal nystatin treatment as it caused significant decrease with all treatments. while vaginal thyme caused significant increase in MCHC.

Table (1): Effect of vaginal candidiasis and treatments

Treatments	NO	RBCs $\times 10^6$ cell/mm ³	Hb g/dl	PCV %
control	8	5.0 \pm 0.3 A	12.0 \pm 0.8 A	33.3 \pm 1.7
DEX 5 mg/kg	8	4.7 \pm 0.5 A	10.3 \pm 0.5 B	34.9 \pm 0.3
Candidiasis 1.5×10^8 cell/ml for 7 days	8	4.5 \pm 0.2 A	9.7 \pm 0.9 B	32.1 \pm 2.9
O Thyme 0.1 ml for 14 day	8	4.9 \pm 0.6 A	10.2 \pm 0.3 B	34.6 \pm 0.19
V Thyme 0.1 ml for 14 day	8	4.9 \pm 0.5 A	12.5 \pm 0.9 A	34.5 \pm 1.3
O Oregano 0.1 ml for 14 day	8	4.8 \pm 0.3 A	10.6 \pm 0.6 B	33.3 \pm 2.3
V Oregano 0.1 ml for 14 day	8	5.2 \pm 0.3 A	10.5 \pm 0.5 B	35.06 \pm 0.4
O Nystatin 0.1 ml for 14 day	8	3.8 \pm 0.6 B	9.8 \pm 0.2 B	30.06 \pm 0.3
V Nystatin 0.1 ml for 14 day	8	3.4 \pm 0.4 B	9.9 \pm 0.1 B	33.26 \pm 0.17

with oils of thyme and oregano compared to Nystatin on RBC, Hb and PCV of mature female rabbits (means \pm SD).

The mean difference is significant at the 0.05 level.

Table (2): Effect of vaginal candidiasis and treatments with oils of thyme and oregano compared to nystatin on WBC, Lymphocyte and granulocytes of mature female rabbits. (Means \pm SD)

Treatments	NO.	WBC $\times 10^6$ g/L	Lymphocytes %	Granulocytes %
Control	8	8.82 \pm 0.9 A	44.7 \pm 12.9 A	49.2 \pm 11.6 A
DEX 5 mg/kg	8	5.18 \pm 0.5 B	64.4 \pm 1.9 B	34.9 \pm 1.13 B
Candidiasis 1.5 $\times 10^6$ cell/ml for 7 days	8	7.18 \pm 1.1 B	66.7 \pm 8.8 B	36.4 \pm 9.2 B
O. thyme 0.1 ml for 14 day	8	9.4 \pm 0.7 A	76.4 \pm 2.0 C	22.5 \pm 1.9 C
V. thyme 0.1 ml for 14 day	8	8.6 \pm 1.4 A	75.9 \pm 3.6 C	30.01 \pm 1.5 BC
O. oregano 0.1 ml for 14 day	8	4.1 \pm 0.8 C	74.36 \pm 7.0 C	30.56 \pm 2.49 BC
V. oregano 0.1 ml for 14 day	8	5.9 \pm 0.2 C	54.88 \pm 23.6 D	34.25 \pm 0.4 B
O. nystatin 0.1 ml for 14 day	8	3.5 \pm 0.5 C	62.63 \pm 28.25 B	37.88 \pm 1.13 B
V. nystatin 0.1 ml for 14 day	8	4.4 \pm 0.2 C	66.44 \pm 0.95 B	32.15 \pm 0.75 BC
LSD		1.617	7.66	11.28

Table (3): Effect of vaginal candidiasis and treatments with oils of thyme and oregano compared to nystatin on platelets, MCV, MCH and MCHC of mature female rabbits. (Means \pm SD).

Treatments	NO	PLATELETES \times 10 g/L	MCV fL	MCH pg	MCHC g/dl
Control	8	193.17 \pm 2.78 A	74.05 \pm 0.81 A	23.65 \pm 0.51 A	31.35 \pm 0.63 A
DEX 5 mg/kg	8	156.83 \pm 7.80 A	73.03 \pm 0.41 A	21.90 \pm 0.37 A	30.22 \pm 0.55 A
Candidiasis 1.5 $\times 10^6$ cell/ml for 7 days	8	196.50 \pm 43.56 A	74.15 \pm 2.07 A	22.35 \pm 1.04 A	29.82 \pm 0.33 A
O.thyme 0.1 ml for 14 day	8	138 \pm 6.89 B	73.93 \pm 0.50 A	22.76 \pm 3.82 A	29.93 \pm 0.83 A
V.thyme 0.1 ml for 14 day	8	474 \pm 64.43 B	72.48 \pm 0.75 A	23.15 \pm 2.82 A	34.67 \pm 4.83 B
O. oregano 0.1 ml for 14 day	8	94.50 \pm 15.97 C	72.73 \pm 2.45 A	20.85 \pm 0.75 A	32.15 \pm 2.73 A
V. oregano 0.1 ml for 14 day	8	534.83 \pm 33.09 D	72.67 \pm 1.05 A	21.05 \pm 0.61 A	31.53 \pm 0.81 A
O. nystatin 0.1 ml for 14 day	8	356.50 \pm 19.23 E	70.60 \pm 1.67 B	22.43 \pm 0.86 A	31.21 \pm 0.92 A
V. nystatin 0.1 ml for 14 day	8	80.37 \pm 1.45 C	77.16 \pm 0.61 C	22.24 \pm 1.78 A	28.20 \pm 3.63 C
LSD		58.66	3.01	N.S.	3.05

The mean difference is significant at the 0.05 level

DISCUSSION

Results of RBC of candidial infected female rabbits showed no significant decrease at ($p < 0.05$) level as well as PCV% values; but shows significant decrease in Hb% only in (table 1) this means that candidial infection can cause anemia of iron deficiency type only this result is coincide with that found by (17,18). Also authors found that treatment with essential oils significantly increases Hb% that significantly decreased by candidial infection; this result is exactly resembles our result of treating candidial infection with both oils of thyme and oregano either orally or vaginally, but in our study treatment with vaginal thyme oil had the better results of increasing Hb%; this is may be due to the content of thyme oil which can be absorbed quickly

from the vaginal mucosa and may has a positive effect on iron metabolism in the animal body and its compounds in addition to being anti candidial agent, may also promotes the health of the animals and the increase in Hb% after treatment may considered as indicating positive potential of probiotics; these results are in the same trends that found by (19) but it did not agreed with them in the results of PCV% when they found significant increase after treatment with essential oils contrary to our result which indicated no significant change in PCV% after treatment with both essential oils of our study.

It is clear also that oral nystatin treatment caused significant decrease ($p < 0.05$) in RBCs and in Hb% (table 1) These results meets with results found by (20) and they may be attributed to the hepatic dysfunction developed by anti-fungal Nystatin (21). As the liver is one of the most important blood forming organ as well as a good iron storing organ which is important in erythropoiesis (22) He also mentioned that those bad hematologic effects of Nystatin after a period of treatment may be also due to its adverse effect on kidneys because the highest concentration of the antifungal was found in the kidney after two hours of administration and this may affect the secretion of erythropoietin from the kidneys tissue which is important for erythrocytic formation which may lead to anemia concerned with low Hb% and PCV% (22). Also (32) found that nystatin caused erythrocyte shrinkage because it triggers cell membrane scrambling and cause at least partially due to entry of extracellular Ca^{2+} and allowing the passage of several ions such as Na^+ , K^+ and Cl^- .

In the table (2) It is clearly obvious that experimental vaginal candidiasis significantly decrease at ($p < 0.05$) level in total leukocytic count and granulocytes but significantly increases lymphocytes;. Adrenocortical steroids used in medicine for their anti-inflammatory and immune suppressive effects (23) So, The decrease in total WBCs in this study may be due to the treatment with dexamethasone before candidial infection as it is clear from the Table(2). But after treatment with oils we noticed clear significant increase in total WBCs and lymphocytes in thyme essential oil either oral or vaginal and oral oregano oil these result were in agreement with result, which confirmed the effectiveness of an anti-inflammatory thyme oil due to strong lipoxigenase inhibitory effects and Because of thymol and carvacrol are the main component of the thyme oil and oregano oils so the humoral immune parameters indicated to elevated in lymphocytes percentage and decrease in granulocytes in blood same results conduct (24) when adding thymol and carvacrol to diet. These results comes in concernments with that found by (25) when he found significant increase in WBCs of male awassi lambs fed *Eruca sativa* essential oil compared to control ones.

So, the present study reach to significant decrease in total WBCs and trend to lymphocytes and granulocytes in groups treated with oregano oil (orally and vaginally) that effect may be due to the effect of *origanum vulgare* pretreatment of the chemotherapy (26) and used in leukemia (27). So, increase in lymphocytes except in group treated with oral oregano that may be due to effect of route of administration on components availability.

While the effect of treatment with nystatin on significantly decreased WBCs than groups treated with two oils these result agreed with (28) Similar results which was found by (20) when drenched *Eruca sativa* oil to male mice and found ameliorative increase of WBCs after treatment with

antifungal agent. Table(3) indicates that candidiasis did not cause any effect on platelets count this means that this type of vaginal infection has no risk of blood clotting, if treating candidiasis with oral oils of thyme and oregano takes places as soon as vaginal candidiasis occurs. In our study treating vaginal candidiasis with thyme and oregano (orally) and vaginal nystatin have decreased platelets count compared with control too, that effect is due to phenols content of thyme oil and oregano oil resulted to possess high antiplatelet activity (29) towards AA and moderate activity towards ADP. Antiaggregant activity was investigated (30) when conduct to the *Thymus vulgaris* is a strongly inhibition thrombocytes aggregation induced by arachidonic acid ,thromboxane A₂ and cyclooxygenase inhibitor. We believed the similarity of phe-

nolic material of (thyme and oregano) oils and also have the same effect of platelet count when treating vaginal candidiasis with oregano oil so the same causes of thyme applied on oregano oil.

So did not treat vaginal candidiasis with thyme or oregano oils (vaginally) and nystatin vaginally for a long period because they significantly increases platelets that may lead to excessive probability of thrombosis and arteriosclerosis (31). we notice in the same study MCV,MCH and MCHC because they affected by the effect of candidiasis and oil treatment on RBCs; Hb and PCV as they calculated from them .The only significant effect on MCHC is with vaginal nystatin treatment which caused significant decrease and this is related to its significant

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