

## **STUDY THE EFFECTS OF TREATING EXPERIMENTAL VAGINAL CANDIDIASIS WITH THYME , OREGANO OIL AND NYSTATIN ON PITUITARY-GONADAL AXIS IN FEMALE RABBITS**

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### **ABSTRACT**

Essential oil of *thymus vulgaris* and *origanum vulgare* were extracted by hexane and analyzed to the component by GC-MAS Gas chromatography. in vivo study was done on seventy two healthy mature non pregnant female rabbits. Animals were divided into nine groups of eight animals for each one. 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, group 5 treated with thyme oil vaginally ,group 6 treated with oregano oil orally, group 7 treated with oregano oil vaginally, group 8 treated by oral nystatin, Group 9 treated vaginally with nystatin. Blood was drawn from animals at the end of the experiment all experimental animals were sacrificed and pituitaries; ovaries and uteri were collected and fixed in 10% formalin for histological technique. result explained that candidiasis caused significant increase in progesterone, LH and FSH also decreased in estrogen compared to control. Treatment with thyme or oregano oils by two routes showed decrease in progesterone and an increase in estrogen than candidiasis group. So FSH and LH decreased in groups treated with two oils compared to candidiasis group but not reached to normal level. Nystatin decreased

all reproductive hormones. results showed improvement of organs structures in treated with thyme or oregano oils

## INTRODUCTION

Vulvovaginal candidiasis(vvc) is the most common infections of females genital canal which is a commonly opportunist fungal disease all over the world (1,2,3). *Candida albicans* lives as normal flora of warm blooded animals and human is a polymorphous fungus or yeast (4,5) . it was isolated in an increased incidence by cultures of cervical mucus of cows suffered from fertility problems (6). Researchs reveals that fluctuating hormone levels resulting from menstruation and pregnancy, as well as the use of oral contraceptives and hormone replacement (i.e., Estrogen therapy), may predispose females to VVC (7,8).(9) postulated that the thyme oil is used to increase libido and as a tonic. (10) reported that they could induce sexual activities in animals by using thyme .Oregano are employed as traditional remedies for menstrual cramps, menstruation and lower stomach cramps related to premenstrual stages (11,12).(13)found that using oregano as natural food additive can support body growth and increase reproductive efficiency of experimental animals. (14) also found that essential oil of oregano can improve developments of embryos during pregnancy when given to pregnant experimental females.

**The aim of study:**To determined the effects of treating vaginal candidiasis with oil thymus vulgaris and origanum vulgare on reproductive hormone and organs in female rabbits.

## MATERIALS AND METHODS

### Preparation of oils Extracts

Oils of dry plant leaves a variable in market was extracted by using hexane as solvent(15) . Essential oils 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. the GC-mass analysis represent the main phenolic compounds contrabuted between thyme oil and oregano oil are: methyl-5-(1-methylethyl)- ; arvacrol ; p-cymen ; antioxine ; isothymol ; karvakrol ;2-hydroxy-p-cymene and 2-Methyl-5-isopropylphenol .

**Animals preparation and management:-**

Seventy-two healthy mature non pregnant female domestic rabbits (*Lepuscuniculus*) were brought from the local markets /Basra, weighing (1400-1800) grams body weight for each one. They were maintained on unrestricted supplies of food that consist of alfa alfa, concentrated pellet ,rabbits were kept for a two week in the animal house of College of Veterinary Medicine / University of Basra, to be acclimatize before using the rabbits for the experiment.

**Candida albicans species:-**

Candida albicans isolated from vaginal mucous membrane of female cows. Inoculums were prepared in concentration of  $1.5 \times 10^8$  cells/ml by comparative with MacFarland solution. Well diffusion method used to determined the antifungal activity by mesurment of inhibition zone of (thyme and oregano ) oil compared with nystatin (16,17,18).

**Experimental design:**

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

**Group 1:** given vaginally physiological saline as (-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 7days as(+ve control).

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

**Group 4:** treated with crude(0.1ml) thyme oil orally for 14 days.

**Group 5:** 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.

**Group8:-** 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 (5ml) of blood is collected from the heart put in EDTA tests tube for the hormonal assay analysis. then animals were slaughtered and organs (pituitaries; ovaries and uteri) were obtained and put in 10% formalin for histological study

**Hormonal assay:**

From bio check, Inc 323 vintage park Dr. Foster city, CA94404.Used progesterone enzyme immunoassay test kit catalog number: BC-1113. And estrogen enzyme immunoassay test kit catalog number: BC-1111. Measurement of serum Follicular Stimulating Hormone (FSH) concentration and serum luteinizing hormone (LH); kit was used Mono bind Inc. lake forest CA 92630, USA).using ELISA- system from bioactive/ Germany.

**Histopathological Technique:**

The ovary, uterus and pituitary of each animal were quickly removed then prepared for histological study according to(19).

**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**

Table (1) shows that experimental vaginal candidiasis significantly increase ( $p < 0.05$ ) progesterone compared with controls. Also the table indicated significant increase of progesterone of oils treated groups of both routs of administration for all oils and nystatin compared with controls but those significant increases did not reach the increase caused by candidiasis. It is clear from the table that highest progesterone increase was due to treatment with thyme oil orally and vaginal nystatin followed by oregano oil treatment but the lowest increase of progesterone was due to oral nystatin treatment. In case of estrogen hormone effects result in the same table showed significant decrease ( $p < 0.05$ ) of estrogen hormone due to experimental vaginal candidiasis but in oil treated groups showed highly significant increase of estrogen hormone ( $p < 0.05$ ) in both routs of treatment of both oils(thyme and oregano) while treating vaginal candidiasis with nystatin decreases estrogen hormone by the two routs of treatment especially in vaginal nystatin. It is clear that treatments with both oils

and by the two routes of treatment caused high significant increase in estrogen compared to control and to result of nystatin treatments .

Table(2) showed significant increase( $p<0.05$ ) in LH hormone due to experimental vaginal candidiasis compared with controls but treatment with both oil of thyme and oregano by the two routes of treatment (oral and vaginal) also caused significant increased in LH hormone level but not to degree caused by candidiasis. The highest significant increase in LH hormone level by oil treatment was due to oil of thyme given by vaginal douching it is also, exceeds the amount of LH hormone caused by candida infection. Oregano oil vaginal treated candidiasis comes at the second degree after vaginal thyme oil treatment. Nystatin treatment also increased LH hormone but not to the significant level compared with controls and less effective significantly than both oils in both routes of treatment. In case of FSH hormone ,the table shows very clear significant increase at ( $p<0.05$ ) level of FSH hormone due to experimental vaginal candidiasis compared to controls, and also it showed a significant increase in this hormone due to both oil treatments (thyme and oregano)in both routes of treatment but values did not reached the value caused by candidiasis. The highest FSH hormone increase by oil treatment was due to oral oregano treatment. The table also indicated statistically significant( $p<0.05$ ) FSH hormone value due to vaginal nystatin treatment compared with control .

**Table (1): Effect of vaginal candidiasis and treatments with oils of thyme and oregano compared to nystatin on estrogen and progesterone levels of mature female rabbits. (means  $\pm$ SD).**

Treatments	NO.	Estrogen pg/ml	Progesterone ng/ml
Control	8	59.33 $\pm$ 2.16 A	0.73 $\pm$ 0.36 A
DEX 5 mg/kg	8	9.31 $\pm$ 1.99 B	0.15 $\pm$ 0.02 A
Candidiasis 1.5 $\times$ 10 <sup>8</sup> cell/ml for 7 days	8	3.38 $\pm$ 0.43 B	9.38 $\pm$ 1.11 B
Oral thyme 0.1 ml for 14 day	8	247.16 $\pm$ 6.85 C	3.10 $\pm$ 0.49 C
Vaginal thyme 0.1 ml for 14 day	8	253 $\pm$ 6.98 C	2.36 $\pm$ 0.87 C
Oral oregano 0.1 ml for 14 day	8	254.16 $\pm$ 11.32 C	2.66 $\pm$ 1.41 C
Vaginal oregano 0.1 ml for 14 day	8	251.83 $\pm$ 8.03 C	2.43 $\pm$ 0.62 C
Oral nystatin 0.1 ml for 14 day	8	31.50 $\pm$ 11.67 A	1.34 $\pm$ 0.48 A
Vaginal nystatin 0.1 ml for 14 day	8	6.88 $\pm$ 1.55 A	3.30 $\pm$ 1.76 A
LSD		22.18	1.93

The mean difference is significant at the(P< 0.05) level

**Table (2): Effect of vaginal candidiasis and treatments with oils of thyme and oregano compared to nystatin on FSH and LH of mature female rabbits. (means  $\pm$ SD).**

Treatments	NO.	FSH IU/ml	LH IU/ml
Control	8	0.15 $\pm$ 0.03 A	0.011 $\pm$ 0.004 A
DEX 5 mg/kg	8	0.25 $\pm$ 0.12 A	0.017 $\pm$ 0.008 A
Candidiasis 1.5 $\times$ 10 <sup>8</sup> cell/ml for 7 days	8	4.77 $\pm$ 0.52 B	1.183 $\pm$ 0.503 B
Oral thyme 0.1 ml for 14 day	8	1.86 $\pm$ 0.04 C	0.106 $\pm$ 0.008 C
Vaginal thyme 0.1 ml for 14 day	8	1.66 $\pm$ 0.40 C	0.45 $\pm$ 0.273 C
Oral oregano 0.1 ml for 14 day	8	1.04 $\pm$ 0.19 C	0.290 $\pm$ 0.067 C
Vaginal oregano 0.1 ml for 14 day	8	1.97 $\pm$ 0.18 C	0.355 $\pm$ 0.282 C
Oral nystatin 0.1 ml for 14 day	8	1.30 $\pm$ 0.16 C	0.136 $\pm$ 0.037 C
Vaginal nystatin 0.1 ml for 14 day	8	1.08 $\pm$ 0.10 C	0.111 $\pm$ 0.016 C
LSD		0.55	0.34

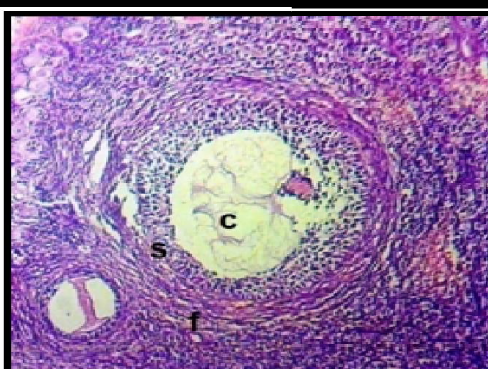


Figure (3) :The section of the ovary of female rabbit infected with vaginal *Candida albicans* and treated with thyme oil orally shows mature Graafian follicles with good granulosa cells and increase stroma cells (S) ,better oocyte cytoplasm (C ) and less fibrous tissue surrounding (f), presence of nucleus (H&Estainx10).

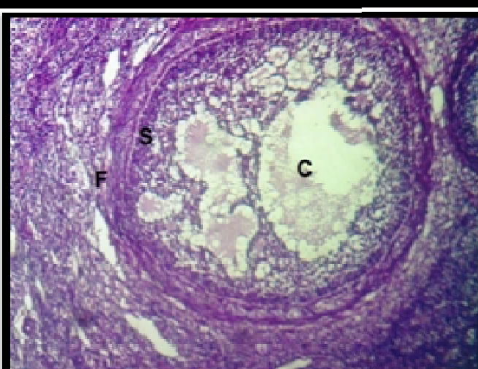


Figure ( 4 ) :The section of the ovary of female rabbit infected with vaginal *Candida albicans* and treated with oil of thyme vaginally shows less improvement of changes in the Graafian follicles than orally treated thyme ,less improvement of oocyte cytoplasm ( c) ,narrow granulosa cells zone and less stroma cells ( s) and thick fibrous tissue surrounding (f) no appearance of oocyte nucleus (H&E stain x10)



Figure (2) : The section of the ovary of female rabbit of control group shows normal structure and active ovary (number of follicle in deferent stage (H&E stain x10 )



Figure (1) The section of the ovary of female rabbit infected with vaginal *Candida albicans* shows Graafian follicles with less granulosa cells less stroma (S) and destructed oocyte cytoplasm (C) and disappearance of oocyte nucleus .the follicles surrounded with thick fibrous tissue (F), (H&E stain x 10).



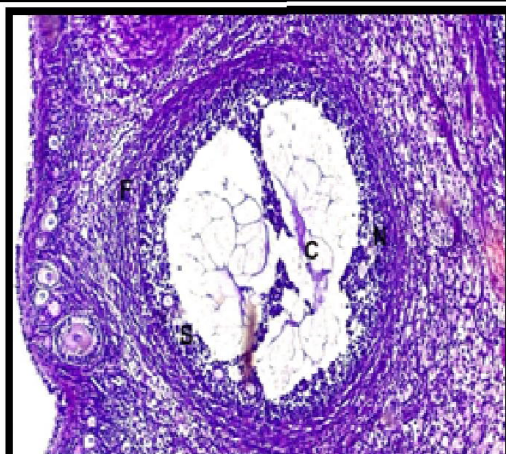


Figure (5) :The section of the ovary of female rabbit infected with vaginal *Candida albicans* and treated with orally oregano oil shows less improvement in Graafian oocyte cytoplasm (c) less stroma cell formation and thick fibrous tissue (f) with newly formed oocyte nucleus (n) (H&E stain x10)

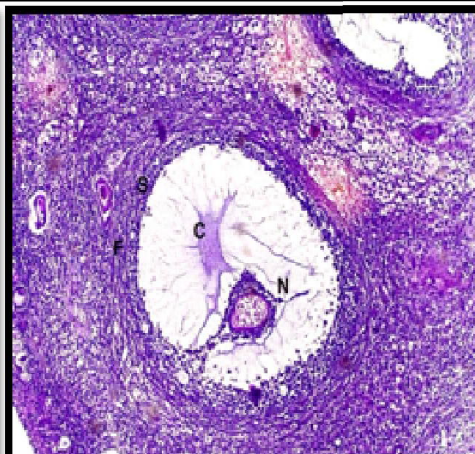


Figure (6) :The section of the ovary of female rabbit infected with vaginal candidiasis and treated with vaginally oregano oil the Graafian follicles shows clear improvement inter oocyte cytoplasm (c) clear granulosa cells and good stroma cell (s) and very clear oocyte nucleus (n) with less fibrous tissue surrounding (f), (H&E stain x10).

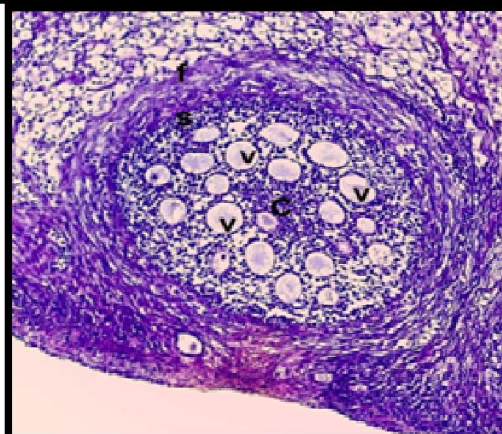


Figure (7) :The section of the ovary of female rabbit infected with vaginal candidiasis and treated with oral nystatin shows no clear improvement in Graafian follicles, multivacuolated oocytes (v), cytoplasm (c) less granulosa cells and few growth of stroma cells (s) thick fibrous tissue surrounding (f) with no clear oocyte nucleus (H&E stain x10)

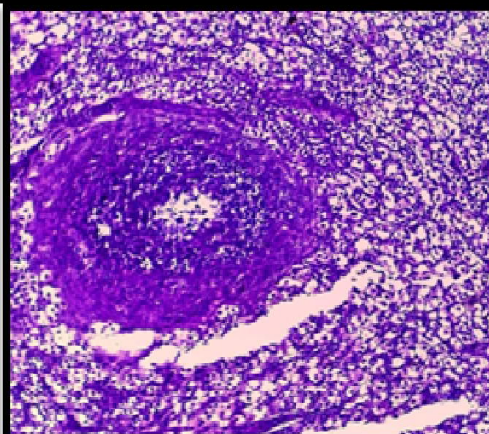
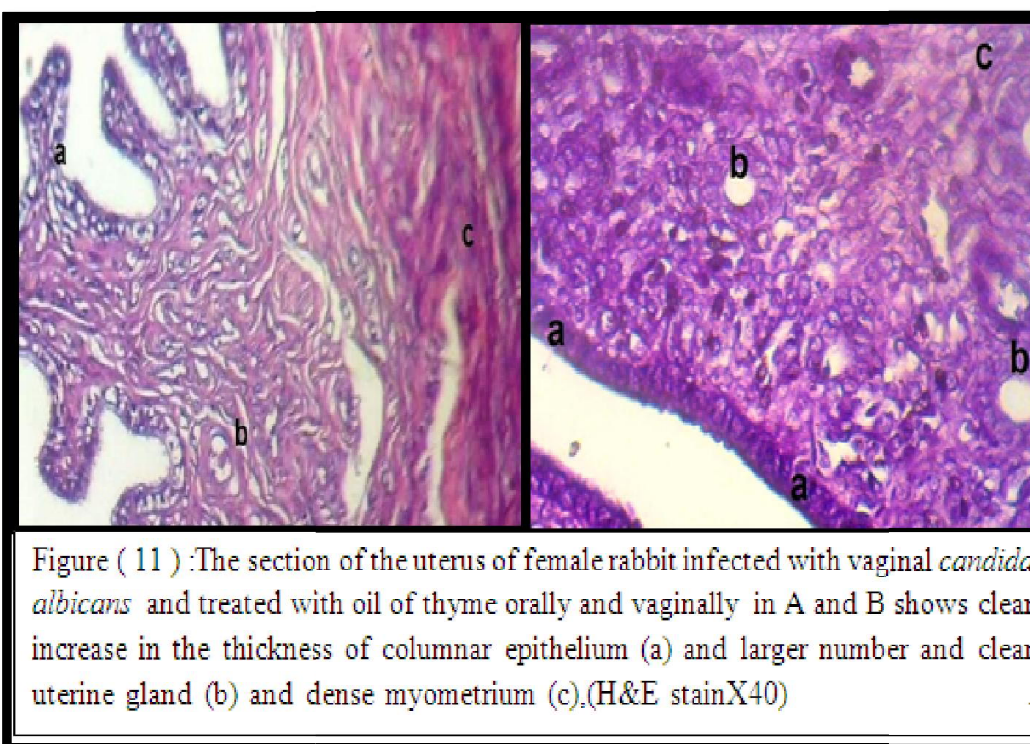
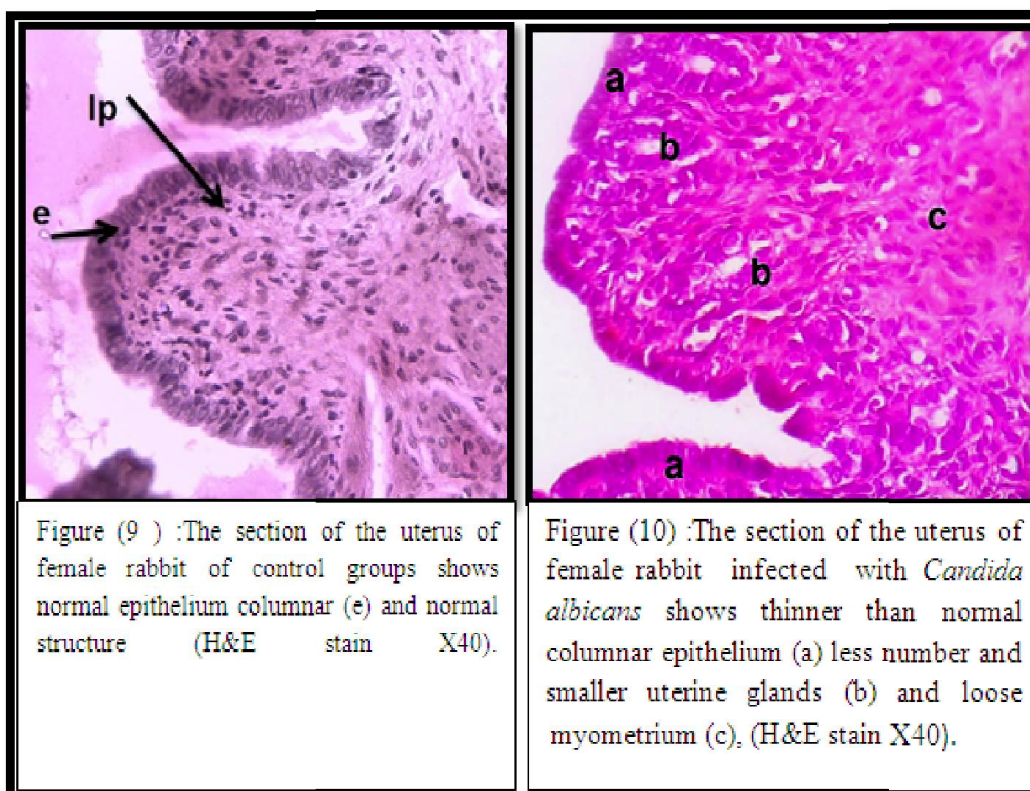


Figure (8) :The section of the ovary of female rabbits, infected with vaginal *Candida albicans* and treated with nystatin vaginally shows calcified graffian follicle (H&E stain x10)







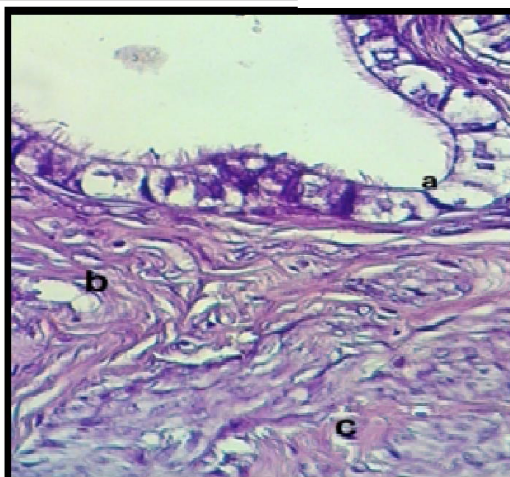


Figure ( 12 ) :The section of the uterus of female rabbit, infected with vaginal *Candida albicans* and treated with oregano oil orally shows less improvement than oral and vaginal thyme treatment ,less development of columnar epithelium (a) less development of uterine gland (b) and loose myometrium (c), (H&E stain X40).

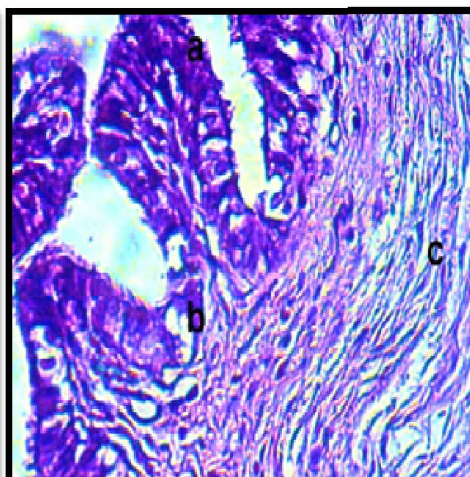


Figure (13 ) :The section of the uterus of female rabbit, infected with vaginal *Candida albicans* and treated with oil of oregano vaginally shows better improvement in columnar epithelium (a) better development of uterine glands (b) and good myometrium ,(H&E stain X40)

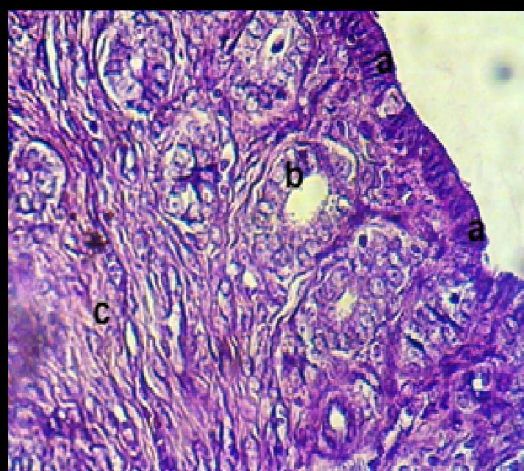


Figure (14 ) :The section of the uterus of female rabbit infected with vaginal *candida albicans* and treated with nystatin orally shows good improvements columnar epithelium(a) and very well development of uterine glands (b) also well dense myometrium (c), (H&E stainX40)

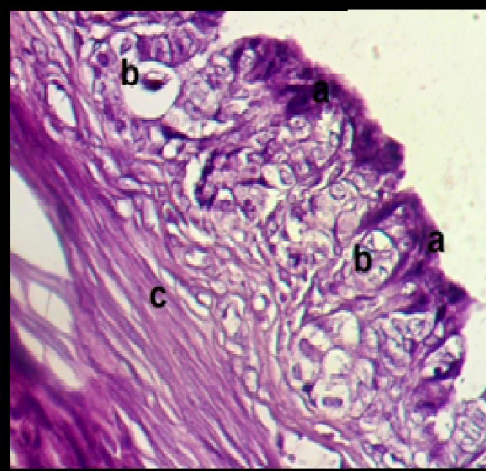
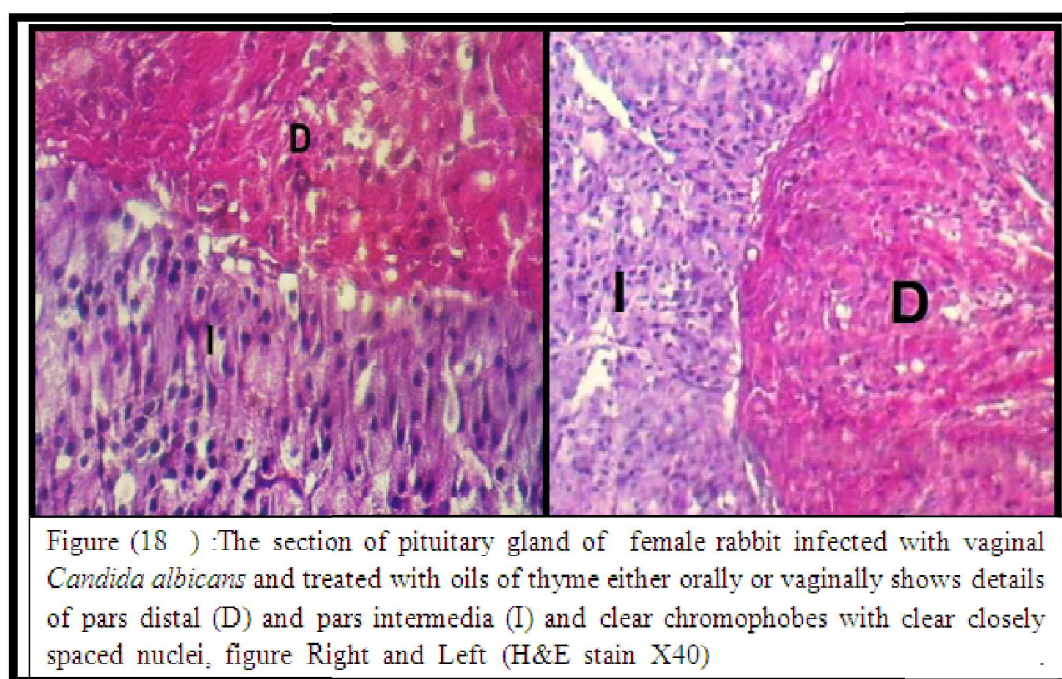
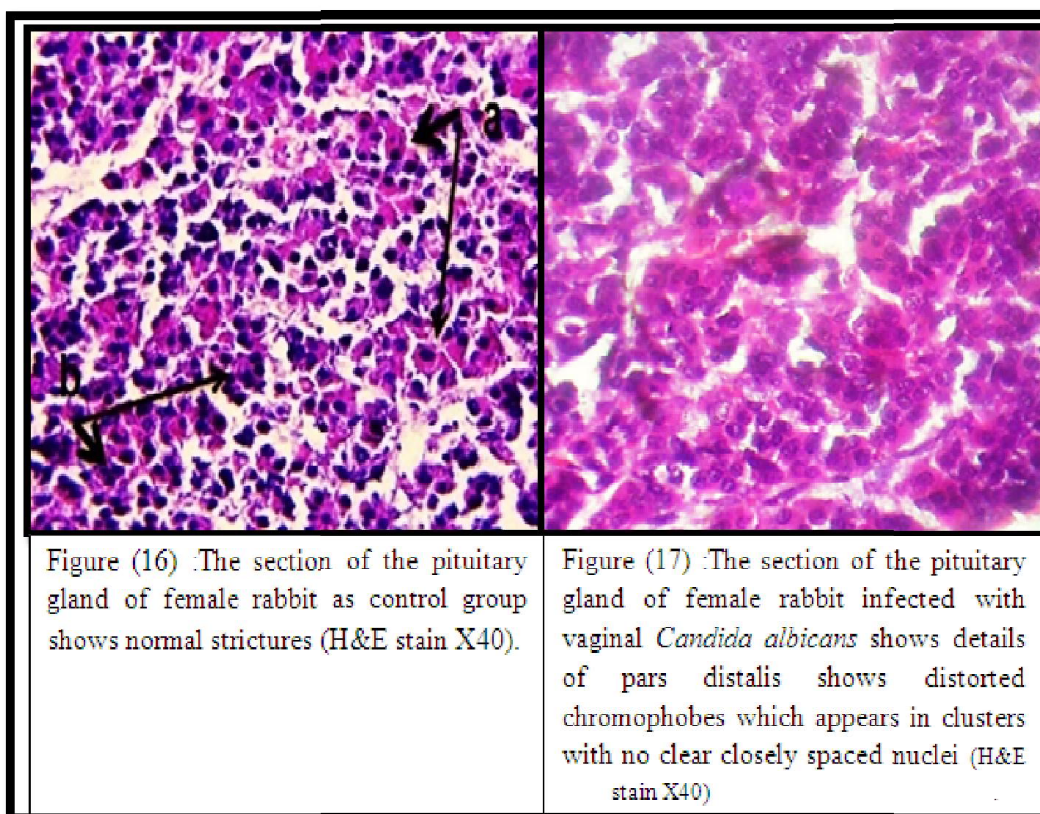
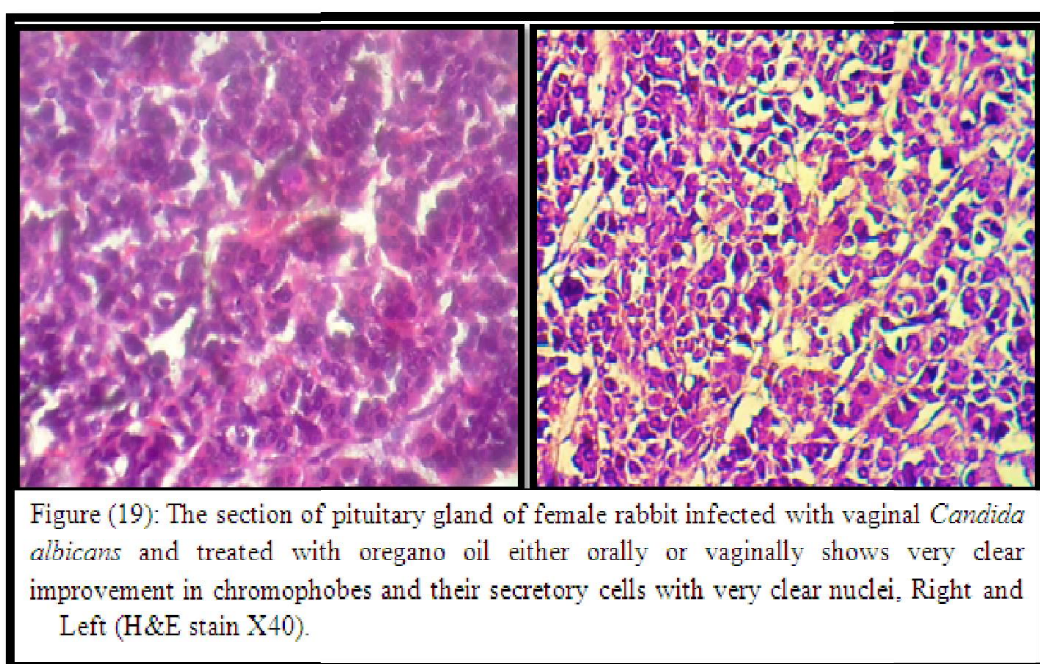
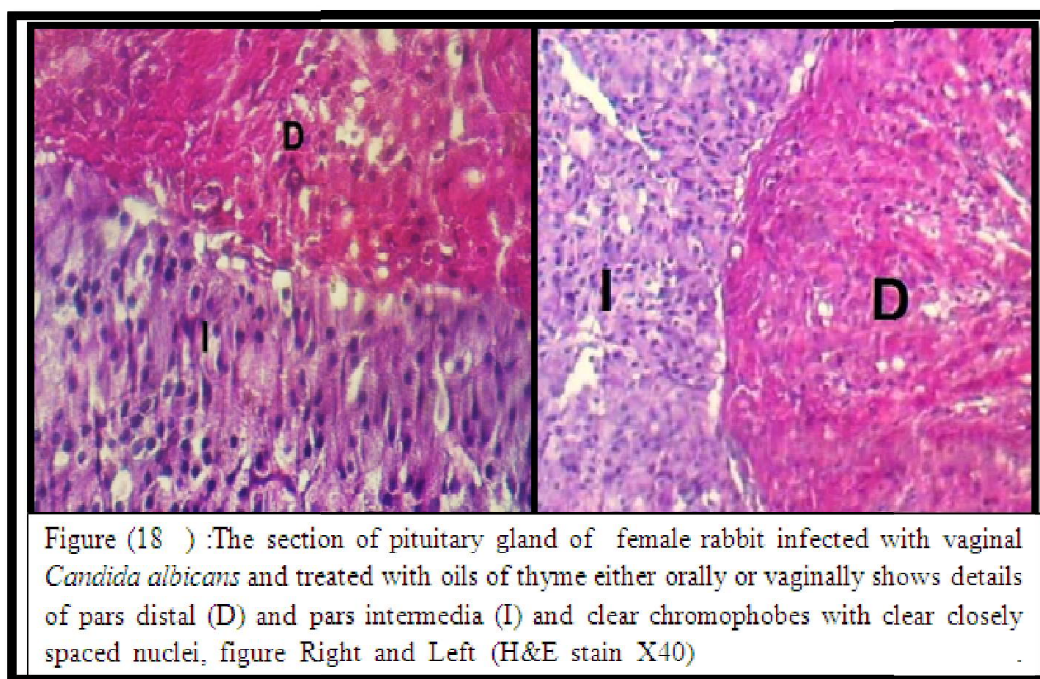


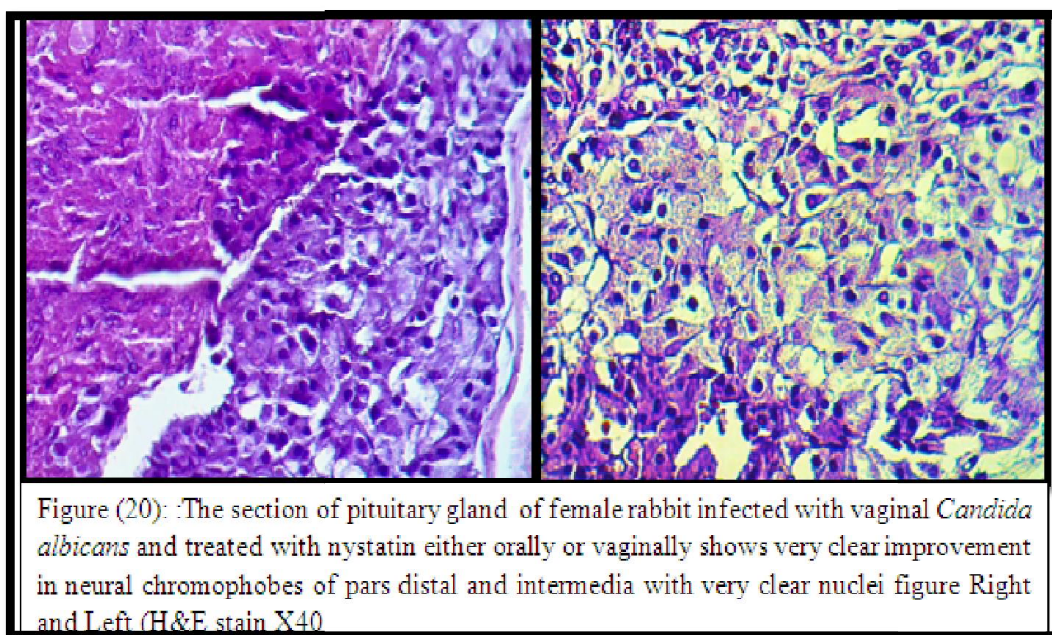
Figure (15 ) :The section of the uterus of female rabbit, infected with vaginal *candida albicans* and treated with nystatin intra vaginally shows clear improvement in columnar epithelium (a) and good development of uterine glands (b) with dense myometrium (c), (H&E stain X40) .











## DISCUSSION

Effect of vaginal candidiasis and treatments with oil of thyme and oregano compared to Nystatin on Estrogen and progesterone levels of mature female rabbits.

Table (1) shows that experimental vaginal candidiasis caused significant decrease ( $p < 0.05$ ) in estrogen hormone this result agreed with that found by (20) but the increase progesterone level due to candidial infection in this study did not coincided with (20) because they found that progesterone did no affected by candidial infection, they said that it may be due to plasma level of progesterone positively correlated and significantly with the agglutination titters of candidail antibodies contrary with high incidence of *Candida* spp during dioestrus (62 %) that may be linked to increase progesterone levels in this phase(21) *Candida*-specific cell-mediated immunity, has been considered the predominant host defense mechanism against mucosal *Candida* infections (22) acting as cellular immunity suppressor, and promoting the expression of a gene that favours synthesis of an epithelial receptor able to bind certain fungi. Also they suggest that low Estrogen production with active vaginal candidiasis suggesting that humoral immunity to *Candida albicans* may be linked to sex steroid hormone especially Estrogen (23) the ovarin figure confirm our result through decreased in zona granulos and followed by absence of oocyte. These results agree with the decrease of zona granulosa of immature follicles. Some studies explained the



granulosa cells of the follicles to exhibited a great number of mitochondria, that might afford a greater developmental potential if the oocytes of such follicles were allowed to mature *in vitro*(24).Accordingly failure of *in vitro*- matured oocytes may be a partly attributed to a reduced number of mitochondria, resulting in insufficient production of adenosine triphosphate required for developmental events (25).These results are similar to that obtained by some investigators, (26)who reported deleterious effects of infection on the reproduction system, i.e., sexual maturation, growth and maturation of the follicles.It is clear from the table (1) that treating vaginal candidiasis with oils of thyme and oregano either oral or vaginal significantly increase both Estrogen and progesterone. This result agreed with result reported by (27);they said that phenolic components of the essential oil affect the hormonal production by pituitary gonadal axis.

They also found that higher doses did not cause any change in the hormones due to negative feedback mechanism on pituitary resulted in no secretion of FSH and LH hormones. Nystatin treatment of vaginal candidiasis in this study decreases both Estrogen and progesterone than candidial infection also less than control significantly this result agreed with that done by (28) when found that ketoconazole has direct inhibitory effects on the ovarian response , including the uptake of the drug into the cells, its intracellular metabolism, and the subsequent inhibition of the steroidogenic enzymes., also our result of estrogen and progesterone reduction due to antifungal drug treatment meets the results of(29,30). Because the structures of the azole fungicides are entirely dissimilar to the androstenedione backbone, the azoles possibly inhibit by binding to a structurally rearranged active site, the 2 other catalytically important sites, or both, in agreement with the kinetics data(31).

Effect of vaginal candidis and treatment with oils of thyme and oregano compared to nystatin on FSH and LH of mature female rabbits. It is clear from table (2) that vaginal candidiasis significantly increases ( $p<0.05$ ) both FSH and LH hormones these results are in agreement with those of (32,33) when they found variations in levels of pituitary gonadotropins (FSH,LH) and their relationship to steroid levels which are well documented and this is may be due to an increase in the level of anticandidial antibodies during the luteal phase over the follicular phase in vaginally infected females with *Candida albicans*.

Treatments with oils of oregano and thyme caused clear significant decrease in both gonadotropic hormones (FSH,LH) than candidiasis these result were coincided with those of (20) the increased gonadotropic hormones in this study may be due to positive feedback mechanism due to low doses of the essential oils and short time period of treatment.(27) because high doses for longer time result in negative feedback mechanism and cause decrease amount of secretion of gonadotropin FSH, LH. (34) The secondary phenol metabolites of plants affect the hormonal networks such as pituitary-gonad axis. Lack of the changes in higher doses may be the result of the feedback regulation of this network on LH and FSH hormones (35) because the oils have antioxidant effect that may lead to the modulation of the hormone concentrations via changing the levels of O<sub>2</sub> in the body and ATP metabolism (36)FSH and LH hormones act synergistically. Also our results of treating with essential oils of thyme and oregano are in accordance with the results of the study performed by (37) on *Coriandrum sativum* plant, it was shown that the hydroalcoholic extract of coriandrumsativum seeds has led to a reduction in serum gonadotropins in the treatment group compared to control group (38). Therefore, the decreased LH secretion can be attributed to extract of the phytoestrogenic compounds (39) Phytoestrogens are natural plant-derived compounds with the structure and function similar to estrogen (40). These compounds result in inhibition of the human and animal gonadotropin secretion and the hypothalamus-pituitary-gonad axis arrest, by affecting the hypothalamic gonadotropin producing cells (41). Also Nystatin treatment of vaginal candidiasis in this experiment caused significant decrease of gonadotropins (FSH,LH) compared to candida infected group and their measurements nearly to reach the control group, this result is agreed with that found by (42).

Experimental infection with *Candida albicans* in this study caused certain histological change in pituitary; ovary and uterus as mentioned in figures(1,10,17).These changes resembles those found by (43) in female mice. Also our results of candidia infection in uterus also agreed with what was found by (44) when they mentioned that *Candidia albicans* can affect uterus when the yeast inoculated in mice and spread through blood stream and cause systemic candidiasis(45). In our study found also clear histological changes in the ovary; and pituitary gland (figure 1,10,17) and uterus but there are no published information or data concerning causes of candidial infections on those organs. In case of treatment

with oils of oregano and thyme our study shows ameliorative effect of all organs included in this study this result agreed with that found by (46) who confirmed the protective effects of oregano against induced damage to organs which might be alleviated by antioxidant activity of oregano and other like such properties of *Oreganum vulgare* and *Thymus vulgaris*; so they preferred to be used as an additive therapy as herbal medicine for fungal infections because they are regarded as a safe supplemental agents against the side effects of certain plants which produce free radical scavenging and increasing activity of antioxidant defence system; so due to infections(47,48).

### دراسة أثار علاج الإصابة التجريبية بالمبيضات المهبلية بزيت الزعتر والمردقوش والنستاتين على محور الغدة النخامية والقند في إناث الأرانب

بشرى فليح حسن ، عبد الرزاق نعيم خضير ، جاسم محمد احمد الكلبى

فرع الفلسفة،كلية الطب البيطري ، جامعة البصرة ، البصرة ، العراق .

#### الخلاصة

تم استخلاص الزيوت النباتية من أوراق نباتات الزعتر والمردقوش بواسطة الهكسان والتي حلت محتوياتها بواسطة جهاز كروماتوغرافيا الغاز. أجريت الدراسة على 72 أنثى أرنب بالغة غير حامل. قسمت الحيوانات الى تسع مجاميع ثمان حيوانات في كل مجموعة. المجموعة الأولى حقنت مهبلية ب 0.1 مل من محلول الفسلجي واعتبرت مجموعة سيطرة سالبة. أكلحت المجاميع الثمانية مناعيا بتجريع الحيوانات 5ملغم/كغم دكساميثازون مرة واحدة يوميا لمدة 7 يوم. المجموعة الثانية اعتبرت كمجموعة سيطرة للدكساميثازون. حقنت المجاميع السبعة الباقية مهبلية بالكانديدا ( $10^8 \times 1.5$ ) خلية كانديدا/مل مرة واحدة لمدة سبع يوم واعتبرت مجموعة سيطرة للكانديدا. المجاميع الستة المتبقية عولجت لمدة 14 يوم مرة واحدة يوميا مع 0.1 مل من الزيوت وحسب كل مجموعة:- (المجموعة الرابعة) عولجت فمويا بزيت الزعتر، المجموعة الخامسة (عولجت مهبلية بزيت الزعتر، المجموعة السادسة) عولجت فمويا بزيت المردقوش، المجموعة السابعة عولجت مهبلية بزيت المردقوش، المجموعة الثامنة) عولجت فمويا بالنستاتين، المجموعة التاسعة عولجت مهبلية بالنستاتين. سحب الدم في اخر التجربة من الحيوانات ثم ضحي بالحيوانات وأخذت منها الغدة النخامية والمبايض والأرحام للدراسة النسيجية. أوضحت النتائج أن الإصابة بالكانديدا سببت ارتفاعا معنويا في هرمون البروجستيرون , FSH, LH و كذلك انخفض الاستروجين مقارنة مع مجموعة السيطرة . العلاج بزيتي الزعتر والمردقوش فمويا ومهبلية خفض البروجستيرون ورفع الاستروجين عن مجموعة الكانديدا. FSH, LH انخفضا في المجاميع المعالجة بالزيتين وبطريقتي العطاء مقارنة بمجموعة الكانديدا إلا أنها لم تصل إلى المستوى الطبيعي. النستاتين خفض كل الهرمونات التكاثرية. أظهرت النتائج تحسن في الأعضاء المعالجة بزيت الزعتر والمردقوش.

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